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CONTRIBUTIONS TO EMBRYOLOGY, No. 15.

# CYCLOPIA IN THE HUMAN EMBRYO.

By Franklin P. Mall.

With three plates and seven figures.

# CYCLOPIA IN THE HUMAN EMBRYO.

By Franklin P. Mall.

The progress made in recent years on the study of teratology has been so marked that it is now possible to reconsider the whole subject and to place it upon a permanent scientific basis. For this progress we are indebted almost exclusively to the experimental embryologists. Problems which formerly seemed impossible of solution—for example, the formation of the double monsters—have yielded as by magic to the embryologist, who made experimental studies upon the living egg. Perhaps the best example that can be brought forward to illustrate this point is the question of the cause of cyclopia. As soon as it was possible to experiment on eggs in such a way that practically all of them developed into cyclopean monsters the explanation of this condition was at hand. For this work we are indebted entirely to Stockard.

Before reviewing the four specimens which I have to report it may be well to give an account of the theories regarding the origin of the cyclopean condition. There are two chief theories, both resting upon an embryological basis. The first of these is that the eggs begin to develop normally and that subsequently, on account of an imperfect development of the head, the eyes coalesce to form a single eye. This theory can be traced back to Meckel. The second is that the eyes arise normally from the midventral line of the brain as a single structure, which in the course of development divides into two eyes. This view was first advanced by Huschke, who believed cyclopia to be due to an arrest of the development of the brain at the time the eyes are forming. Although Huschke's opinion seemed to be quite sound at the time it was advanced, it did not attach itself firmly to literature, nor could we well accept it at present as resting upon a sound embryological basis. The figures which he gives in illustration shows first an early stage of development of the brain, with a marked forebrain, and then an embryo with two eye-vesicles hanging to the forebrain. He apparently confounded the whole forebrain with the eye primordium.

Meckel's studies rest upon much sounder embryological and anatomical evidence, and his views gradually made their way into the literature of teratology. Until a decade ago it was practically impossible to find any description of cyclopia in which Meckel's studies were not reflected in the background. According to Ahlfeld, Meckel states that cyclopia is characterized by a coalescence of the eyeballs as well as of the orbital cavities. In case the orbital cavities unite very early in development they distend evenly in a lateral direction. The tissues which normally separate these cavities are absent or are pushed aside. In fact, the structures which give the frame to the nose are most rudimentary, or absent, while the nose itself is represented as a membranous snout, varying in form and located above the confluent eyes. The mouth is frequently involved in this type

of monster and is usually rudimentary, while in some instances it, as well as the snout, is missing altogether. Since the eyeballs are developed from pouches which arise from the forebrain, it follows that the primary cause of this anomaly is not to be sought in the development of the skull, but in the development of the brain itself. We find in these cases that the width of the forebrain and midbrain diminishes in the course of their development, corresponding to the union of the orbital cavities and the eyeballs, making the brain appear at term much like that of an embryo of the twelfth week. In addition to the atrophy of these parts, the formation of the hemispheres as a single body is especially noticeable—that is, they have not been divided into two lobes. This division often is only slightly indicated. The ventricles have united to form a single large cavity. In most of the cases at birth the quantity of fluid within the ventricle is increased, so that as a rule we have a large, bladder-like body in place of the forebrain. In this way is the fact explained that in spite of the rudimentary development of the brain there is no diminution of the size of the fore part of the head, as most cases of cyclopia are accompanied with hydrocephalus. There are cases, however, in which there is no hydrocephalus, which naturally result in a small head. This is most pronounced in cases of cyclopia in double monsters in which the head contains two brains. In these cases a symmetrical development of the brain is very rarely found. The rudimentary brain can no doubt be held responsible for the most pronounced specimens of cyclopean faces. In may be taken for granted that the nerves which are to supply the deformed eye and face are simple in their development, corresponding in amount with the degree of the anomaly.

This general description of the anatomy of the eye and face in cases of cyclopia is one which will be found in most teratologies, and in all of these accounts it would appear as though the authors mean to say that the eyes must arise from the forebrain and that they subsequently unite into a single compound eye, more or less hourglass-shaped, due to an arrest of the growth of the brain which in some way interfered with the development of the forehead and eventually left the nose above the cyclopean eye. Teratologists are inclined to believe that the accompanying hydrocephalus is to be viewed as the primary cause of the anomaly, although in many instances they try to trace this back to amniotic bands, which, however, are not found in human specimens of cyclopia, and of course such bands could play no rôle in the formation of this anomaly in animals which develop without an amnion. Furthermore, the explanation of the formation of monsters by means of amniotic bands is alluded to in recent teratologies only as one of the myths of teratology.

In my paper on monsters some ten years ago I gave a review of the experimental work upon cyclopia as it appeared at that time. These statements I shall recapitulate in part in order to bring out more clearly the recent progress made in the study of cyclopia.

In numerous experiments upon frog's eggs, Born, in 1897, occasionally produced monsters by splitting the head through its sagittal midplane after the medullary plate was formed, and then readjusting the two halves. The pieces

united at once, but in a few instances a double eye was formed. Later Spemann, making similar experiments, also produced cyclopean embryos. In some of Spemann's experiments triton eggs were ligated in the sagittal plane during segmentation, and frequently embryos with double heads resulted, one or both being cyclopean. Spemann believes this experiment proves that in its differentiation the cyclopean eye is defective from its beginning and is not produced by concrescence of two eyes which started to develop normally. Levy also produced cyclopean monsters by cutting off the front of the head of triton larvæ. In the course of two weeks the two eyes approached each other and formed a double eye, but they did not fuse. However, the pigment layer was destroyed, or absent, at the point of contact. The two optic cups touched each other, but did not unite.

In 1906 Harrison produced a new variety of cyclopia by removing the entire brain from frog embryos. In these specimens the eye moved to the back of the head and appeared to unite in a single vesicle in the region usually occupied by the pineal eye. These specimens are still unpublished.

By pricking the extreme anterior end of the embryonic shield in *Fundulus* eggs, Lewis found that many of the eggs developed into cyclopean monsters. All grades of defective eye were formed—from a double eye and hourglass-shaped eye with two lenses to oblong eyes with two lenses or with but a single lens. The optic cups blended absolutely, thus apparently showing the mode of development of these eyes. Lewis also found that in many of the embryos the brain had not been injured at all, but that the prick had destroyed the nose only. This experiment seems to show conclusively that it is the absence of tissues between the eye primordia which allows them to come together and unite, and that a rudimentary brain is unnecessary.

In his remarkable experiments on the artificial production of a single median eye in the fish embryo by means of sea-water solutions of magnesium chloride, Stockard found that 50 per cent of the embryos developed cyclopia. In these embryos the optic cups were fused at an early developmental stage, much as was the case in Lewis's specimens, in which the embryonic shield had first been pricked. The union of the two cups formed a large compound eye, which in turn derived its lens from the epidermis immediately overlying it in the midline of the embryo. How the magnesium acts upon the embryo is not clear from Stockard's description. No doubt it will be found that it retards the growth of the frontal process, much as in Lewis's experiments. The salt, however, acted upon the whole body of the embryos, for their development was retarded, thus making them smaller than usual, and their circulation was feeble, but they did not die. In these embryos, as in Lewis's specimens, the growth of the brain was normal. The remarkable experiments of Stockard set at rest all germinal theories of cyclopia and prove that every egg has in it the power to develop cyclopean monsters.

These experiments, as well as the numerous pathological embryos with deformed heads and faces which I have studied, prove at any rate that in the formation of many monsters there is an extensive destruction and shifting of tissues. This is also well illustrated in the production of club-foot in the human

embryo. It has frequently been noticed that tadpoles whose growth has been arrested develop stubby or club tails and fins—a condition which corresponds well with club-shaped extremities in man. Our collection contains 18 embryos with deformed legs and feet, with catalogue numbers less than 400, ranging from the very earliest period until the fetus is well formed. The leg-buds are irregular in shape and are filled with condensed mesenchyme; sometimes they are stubby on one side of the body and normal on the other. The study of the larger embryos shows that there is a variety of inflammation of the tissues which is especially well marked in the tendons and around the cartilages. In general this condition may be accounted for by an arrest of development due to impaired nutrition. At any rate, embryos that are not developing well—experimental larvæ and human embryos with other malformations—often have club-shaped arms, legs, fins, or tails.

The inference to be drawn from the above summary is that after the eyes have become well formed they do not pass out to the side of the head as in normal development, but approach each other and more or less unite, and thus form cyclopia. Recent embryological studies of Stockard and of Spemann show conclusively that this view can not be correct, for it is found that the cyclopean condition can be followed back through earlier and earlier embryos, and that all varieties of cyclopia are present while the eyes are still firmly attached to the brain. It is now maintained by Stockard that, from its very beginning, the eye primordium is in the midventral line of the brain, and that in cyclopean embryos there is an arrest of its development, the eye remaining median or dividing in part, forming the hourglass-shaped cyclopean eye with two lenses, etc. This view is combated more or less by Spemann; but I must confess that it is difficult for me clearly to understand his view as given in his various papers.

Through his well-known magnesium experiments, Stockard has been able to procure an abundance of material for the study of the early development of cyclopia. He proves first of all that the condition of cyclopia is present in the earliest stages in which it would be possible to recognize it. At no stage are there two normal eyes which subsequently blend to form a single eye. The cyclopean condition is present in the eyes while they are still closely attached to the brain. Stockard observes, secondly, that the cyclopean eye is rarely equal in extent and size to the sum of two normal eyes combined. A cyclopean eye is, as a rule, very slightly if any larger than one normal lateral eye, and in fact it is often much reduced or actually minute in size as compared with a normal eye. According to Stockard, this fact indicates most decidedly that the eye material, as such, has been injured or arrested in development and differentiation. He believes that we are scarcely warranted in assuming, as have various authors at different times, that cyclopia is due to a fusion of the eyes after they have arisen from the brain and that the earlier in development the fusion occurs the more intimately associated the two eye components become. This view, according to Stockard, has been proved incorrect by actual observation on cyclopean monsters, where it is found that the cyclopean condition of the eye—whether large and hourglass-shaped or of small size resembling a normal eye—is present from the earliest

appearance of the optic vesicle from the brain. In other words, the several degrees of the cyclopean eye come off from the brain in their final condition.

The idea of the fusion of the eye parts, Stockard continues, was deep-rooted, however, and exists in the recent views of Spemann in a refined form. Spemann believes, as others have previously suggested, that cyclopia is due to an absence of non-ophthalmic tissue in the median region of the medullary plate or groove. This lack of median tissue allows the eye primordia, which he holds to be lateral in position, near the borders of the medullary plate, to come together and fuse in the median plane and later give rise to a cyclopean eye. Cyclopia, according to this view, occurs in a more or less passive manner, and is actually a fusion of the eye primordia of the two sides during development. Stockard adds that he is certain that this fusion explanation, which has now been forced entirely back into the medullary plate, is as false as its bolder predecessor, which assumed the fusion to take place outside of the brain-tissues after the optic vesicles or cups had arisen. He says that Spemann did not at first advocate this late-fusion view, but claimed (from his experiment on *Triton*) that the cyclopean eye arose out of the medullary tissues in its final condition; subsequently, however, he assumed the rôle of a most ardent supporter of the view that the fusion of the optic primordia takes place within the medullary plate.

It may be added that there is no known instance of the formation of cyclopia by experimental methods after the eyes are fairly well formed in normal development. All the experiments in which cyclopia has been produced were made upon the embryo at a stage before the eyes could be recognized under the microscope. One must recall that Stockard's magnesium experiment is effective only when it is done before the embryo is 15 hours old. In fact, Stockard found that the best results were obtained if the eggs were placed in magnesium-chloride solution immediately after fertilization. If the eggs are not placed in the solution until 15 hours after fertilization, before the germ-ring forms and begins its downward growth from the yolk-mass, no cyclopia occurs. Cyclopia is less frequent in eggs which are treated at later stages than in eggs immersed in magnesium-chloride solution during the fourth and eighth cell stage. It appears, then, that the critical stage at which cyclopia is best produced with magnesium is shortly before the germ-ring is formed. According to Stockard, a 15-hour embryo has the germ-ring beginning to form and descend over the yolk-sphere; the embryonic shield is scarcely indicated, but appears soon afterward. Embryos of later stages subjected to the same treatment develop normally, or at least do not show cyclopia, while embryos younger than 15 hours, and at as early a stage as the first cleavage, are much more readily affected in such a manner as to cause the cyclopean defect. The optic vesicles appear at about 30 hours after fertilization, but the stimulus must be applied at a time sufficiently long before this process occurs, since a number of important steps in eye formation are doubtless taking place before the visible signs of optic vesicles are present.

It is interesting to note that the Lewis pricking experiment is made at a stage in which cyclopia can no longer be produced by placing the eggs in a magnesium

solution. According to Lewis, the experiment should be made on the second day. Although he does not give his experiment in hours, his illustrations show the stage of development. According to these the embryonic shield is well formed. The experiments of Lewis were first described in my monograph on monsters, but they have since been reported in detail by their author. As has been stated, Lewis produced cyclopia in *Fundulus* by pricking the middle of the anterior end of the embryonic shield two days after fertilization. In the course of a few days it became apparent that in some of the eggs operated upon the eyes had developed normally, while in others they had become cyclopean. Most of the specimens were killed after 15 days. Pricking of the embryonic shield was accompanied by the escape of a slight amount of tissue, and as there is little or no regeneration of the central nervous system in *Fundulus*, the defect caused at the time of pricking subsequently became more and more apparent as development proceeded. Both Lewis and Stockard have found that cyclopean *Fundulus* embryos usually develop with a normal brain, thus no doubt accounting for the vitality of this special cyclops. Furthermore, it appears that the eye primordium in *Fundulus* is more circumscribed than in many other animals.

In a number of his experiments Lewis found that the material withdrawn with the needle-point came from one side of the anterior end of the embryonic shield, with a resulting abnormality of the eye on that side. In a specific case, at the time of hatching, the right eye of the specimen consisted of a small bit of retina connecting with the otherwise almost normal brain-wall. The left eye was apparently normal, as were also the brain and the nasal pit. In other specimens, in which the operation was about medial and was done at the time the embryonic shield was beginning to form, the embryos developed with the two eyes in contact, with two optic nerves and two lenses. Among other specimens there is one with a cyclopean eye, having a layer of pigment narrowing between the two eyeballs. In specimens operated upon at a little later stage there is a median cyclopean eye with two lenses, one pupil, and one cup cavity. Using Lewis's language, the large optic cup shows in sections a very beautiful median eye with complete continuity of the layers of the retina of two components about a single large cup cavity of a single lens.

According to Lewis, the explanation of these various abnormalities is in a way comparatively simple, if we assume that in the early embryonic-shield stage the various parts of the central nervous system and the eyes are probably already predetermined, and, secondly, that there is very little or no power of regeneration in this tissue. Numerous experiments on regeneration indicate very clearly that there is little or no regeneration of the tissue (at least of that of the central nervous system) extruded at the time of the operation. The repair which takes place after the operation consists merely of a rapid closing together of the parts left remaining, and thus a healing of the wound occurs without regeneration of lost parts. This closing of the wound is accomplished in a few minutes, and primordia are thus brought into contact which normally are quite widely separated—those of the two eyes, for example. The subsequent differentiation adjusts itself to the

new relations of these primordia with the resulting abnormal forms. Thus, as one examines these developing embryos, from the time the eye primordia are first visible in the living specimens under the binocular microscope, they appear to have the same amount of fusion or loss of eye that is clearly to be found in the same individual at later stages and at the time of hatching. So we can explain these cyclopean forms by a fusion of the primordia of the two eyes immediately after the operation, even though at this time no primordia are visible. Differentiation of the eye-tissues evidently occurs some time before it is visible by our crude microscopic methods.

Briefly summarizing the experiments of Stockard and Lewis, it may be said that Stockard produced cyclopia by immersing *Fundulus* eggs in a magnesium solution before the formation of the germ-ring, while Lewis operated upon the embryonic shield after it had arisen from the germ-ring. According to Stockard, the magnesium solution possesses a decidedly anesthetic effect and inhibits the growth of the optic out-pocketings; and therefore the condition of cyclopia must be present before the formation of the optic cup—which he believes to be median—the anesthetic effect preventing the medial cup from dividing, thus bringing about the cyclopean condition. According to Lewis, the optic primordia are brought together through the removal of a small amount of tissue which normally lies between them. The primordia then unite and produce all degrees of the cyclopean condition. For practical purposes either theory will suffice to explain the condition as found in man, and there is at present no evidence which can decide which of the two is correct, for I may add that (as Dr. Lewis informs me) the optic primordia arise very close to the ventral midline of the brain, being separated by only a few cells.

Stockard has recently attempted to define more accurately the eye primordia in *Amblystoma* by operating upon the medullary plate. First of all, he found that pricking the medullary plate, as Lewis pricked the germ-shield in *Fundulus*, had no effect whatever upon the growth of the eyes. They invariably grew in a normal way. He then removed various parts of the medullary plate and found that the removal of a median strip about one-fourth to one-third the width of the medullary plate resulted in eyeless embryos. The entire eye primordium apparently lies within this median strip. When a narrower strip was removed the embryos developed with one eye, with defective eyes, or with no eyes at all. From these experiments he concludes that the primordia of *Amblystoma* arise in the anteromedian portion of the medullary plate, and not from two independent primordia, as is believed by Lewis.

It may be added that the earlier papers of Lewis and of Stockard were written partly to demonstrate that cyclopia is not an hereditary but an acquired quality. This opinion is much at variance with that of Wilder, who upholds the hereditary theory. In this relation may be stated that there are two records of cyclopia in twins. One, by Ellis, is referred to by Ashfeld on page 283 and is also illustrated in figures 11 and 12, plate 47, of his Atlas. The other is by Van Duyse, and is referred to by Schwalbe and Josephy on page 210. The Van Duyse case is inter-

esting, as both parents and grandparents were perfectly healthy and monsters were not known to have occurred in the family. The mother had been pregnant eight times, four of the pregnancies ending in abortions. The first child had harelip, the second had cleft palate, the third was normal, and the fourth pregnancy resulted in the cyclopean twins. In my own experience I can report an even more remarkable case. In 1900 a pig uterus was brought to me which contained a number of normal embryos and three cyclopean embryos, all of about the same stage of development. The cyclopean pigs measured about 40 mm. in length, and each of them had a marked depression in the front of the head and a single pigmented eye with a snout over it. Unfortunately I did not keep the uterus of this specimen, so that it was impossible for us to examine it with care.

The somewhat lengthy discussion on the differentiation of the eye from the medullary plate is justifiable, because at least one of the specimens I have to report is practically a perfect one, which enables me to discuss the origin of the cyclopean eye in a somewhat connected way, from the condition found in normal embryos. After a description of this specimen I purpose to compare the eyes and brain with the same structures in several younger embryos in the Carnegie collection, as well as with those found in the literature. I shall begin with the smallest specimen to be described, namely, No. 559.

### Embryo, CR 6.5, Normal in Form, with Cyclopia, Carnegie Collection, No. 559.

This interesting specimen was sent to me by Dr. Merrill, of Stillwater, Minnesota, on December 21, 1911. Dr. Merrill writes that the specimen came from a white American, who is the mother of one child, 9 years old. The patient gives a subsequent history of three of four abortions which took place early in pregnancy. The last menstrual period before the present abortion occurred on October 27, 1911. The abortion followed on December 20. No particulars are obtainable to account for the abortion and there is no evidence of its having been produced by mechanical means. The patient has a history of irregular menstruation and has been treated for metritis and endometritis. It is impossible to obtain any history of venereal disease.

The unopened ovum, measuring 20 by 15 by 12, came to us fixed in formalin. It is almost entirely covered with villi which branch three of four times and are about 3 mm. long. On one side there is a small area without villi, covered only by the transparent chorionic membrane. Through this can be seen a well-formed embryo, apparently normal, measuring about 8 or 9 mm. in length and filling about one-half of the ovum. The remaining half is filled with dense reticular magma. The umbilical vesicle is spherical, measuring about 3.5 mm. in diameter. The appearance of the ovum before and after opening is shown on plate 2, figures 2 and 5. The embryo was removed by cutting the umbilical cord near its attachment to the chorion. Photographs were then made at both sides of the embryo, at one diameter enlargement, care being taken to get the exact profile pictured. Numerous other photographs were taken, and it then became apparent that we were dealing with an embryo with a very curious deformity of the head. We

also made profile outlines of the two sides of the specimen, being careful to have them in geometrical projection. The branchial region of the two sides of the head were then very carefully drawn. These drawings are reproduced in plate 1, figures 2 and 4. The specimen was dehydrated by placing the entire specimen in several grades of alcohol. It was opened a year later, January 1913, at which time the photographs were made. The specimens were prepared for embedding in February 1914. At this time the embryo measured in absolute alcohol GL 8.6 mm., CR 6.5 mm. In xylol the GL measurement was reduced to 8.2 mm. It remained in several changes of xylol for 30 minutes and then was rapidly transferred through several dishes of paraffin, the entire time of this operation being one hour. The aim was to embed the specimen as quickly as possible in order to avoid any undue shrinkage. It was then cut into transverse sections 20$\mu$ thick. These were stained upon glass slides in hematoxylin and Congo red. It was now found that we had an unusually good series of practically prefect sections, none of them being distorted. Many cell divisions were found in the tissues of different parts of the embryo. We now readily realized the condition of the head, as we found in the sections a perfect series of an exquisite cyclopean embryo. A month later the half of the chorion which was removed to expose the embryo was also cut into serial sections 20$\mu$ thick and stained with hematoxylin and eosin. Further examination of the part of the chorion from which the embryo was peeled showed the amnion close to the chorionic wall, at the point where the cord had been cut. At the point of juncture there is no complete cord, but in its place are numerous single blood-vessels, making the chorionic attachment of the cord appear like the hairs of a camel's-hair brush. This part of the chorion was now stained *in toto* with alum cochineal and cut into serial sections, in order to determine the exact nature of the tissue of the cord as it spreads out into the chorion; for the anomaly here seen had not been encountered by us before.

A superficial survey of the villi of the chorion shows it to be apparently normal, but the interior of the ovum, on account of the great amount of magma encircling the embryo, indicates that the ovum is pathological. Furthermore, the chorion is much too small for a normal embryo of this size. As a rule, pathological embryos are contained in ova which are larger than normal. The attachment of the cord to the chorion is also anomalous and a superficial glance at the head of the embryo shows that it is atrophic. The whole front of the head is occupied by the midbrain. There is no lateral bulging to correspond with the cerebral vesicle. The small pigmented eyes are buried deeply in the head and there is a pronounced frontal process in front of them. On the right side of the head, just in front of the eye, was a small protruding villus-like body which subsequently proved to be the snout (plate 1, fig. 2; plate 2, fig. 7; and plate 3, fig. 5). A more detailed description of the anatomy of the eye region will be given in considering the sections of this specimen. In order to interpret the sections properly a plaster-of-paris model of the brain and head was made at a scale of 50 diameters. Later it was found that this model was on too small a scale to include the muscles of the eye, and a second model of the eye region was made at a scale of 100 diameters.

The two halves of the chorion having been cut into serial sections, it was possible to ascertain with greater precision the attachment of the umbilical cord as well as the amniotic adhesions spoken of above. It was found that the cord was attached to one half and that there were delicate amniotic adhesions in the other half of the specimen. The attachment of the cord was by means of blood-vessels passing directly from the embryo to the chorion, while the adhesions in the second half were by means of loose strands of mesenchyme cells binding the amnion to the chorion.

The tissue of the chorion appears much like that in normal ova. It is of about the same quantity and is possibly a little more fibrous. The mesenchyme of the villa is well infiltrated with embryonal blood-cells, and their trophoblast is quite scanty. At points the villi are stuck together with maternal blood, in which are found islands of syncytial cells, showing that the infiltration of blood took place before the time of the abortion. The mesenchyme of a few of the villi takes on an intense hematoxylin stain, which indicates that it is degenerating. In fact, the cells of the mesenchyme in many of the villi have mostly disintegrated.

The reticular magma stains intensely with eosin. Scattered through the dense network composing it are numerous large protoplasmic cells containing nuclei. In certain places the cells accumulate in large masses, forming colonies. No doubt these are the so-called Hofbauer cells, so well described by Essick.

The embryo had been cut into a very perfect series of transverse sections, which show that very little unequal shrinkage took place while it was being embedded. Nearly the entire wall of the central nervous system is in apposition with the surrounding mesenchyme. However, there are occasional separations along the dorsal midline in the head region, the most pronounced one being around the midbrain, but this is not marked. The thin roof-plate of the hindbrain has collapsed only to a slight extent. All in all, the preservation, embedding, mounting, and staining of this specimen is quite perfect.

There are numerous cell divisions in the brain-tube around the central canal as well as in the retina of the cyclopean eye. Furthermore, these cell divisions are found also in the atrophic cerebral vesicle, showing that at the time of the abortion the atrophic cerebral vesicle, as well as the cyclopean eye, was growing actively.

The form of the head is well shown in the figures. In order to study the head with greater care a model was made of the external form at a scale of 50 diameters. This model shows the shrinkage of the head while the specimen was being embedded. It was made so that the entire head could be removed from the body in order to give a face view of the embryo, which could not be obtained from the specimen before it was cut into sections (plate 1, fig. 3). The model also shows the midbrain to be very prominent, the frontal process being pronounced but small. The eyes are shown deep in the head, and the snout protrudes above the mouth from a point immediately below the process and just in front of the eye. The body otherwise appears to be normal in form, and a microscopic survey of the sections shows that the tissues are also normal in structure.

The lateral view of the head as given in the illustrations on plate 1 may be compared with a normal embryo of the same size; for this purpose I will take the Huber embryo No. 3, pictured by Streeter in figure 86, Volume II, of the Manual of Human Embryology. In the Huber specimen the floor of the forebrain is unusually large, as may be observed by comparing the above-named figure (86) with Streeter's figure 83, which is taken from His's embryo $Br_3$. This region is also somewhat smaller in the Huber embryo than in a model of the brain of our No. 163 made by Dr. Lewis. No. 163 is 9 mm. long, and a profile drawing of its brain is shown in figure 428 in the second volume of the Manual. Careful comparison of the model of the brain of the cyclopean embryo No. 559 with models of the brain of normal embryos shows clearly that there is a decided ventral median defect of the brain of this embryo, from the mammillary bodies to the front end of the brain. This defect naturally takes away the tissues between the eyes and between the cerebral vesicles. In other words, the floor-plate, as shown in figure 55 in the Manual, reaching from the mammillary bodies to the neuropore, has been cut out. In the cyclopean specimen the hypophysis is also absent. The eye-stems have been taken out, the olfactory lobes are absent, and the brain is reduced to a single vesicle, as is the case in older specimens of cyclopia. Such an extreme destruction of the base of the brain rarely occurs in cyclopean *Fundulus* embryos, as in this animal, according to Stockard and Lewis, the brain is frequently entirely normal, the eyes alone being deformed; but in man marked brain defects have always been found to accompany cases of cyclopia.

The optic vesicles in No. 559 form an hourglass-shaped body with two lenses, as shown in plate 1, figure 1, and plate 3, figure 4. The tissues are beautifully preserved and apparently normal in structure. The primary chambers of the eyes communicate freely with each other (plate 3, fig. 4), and through a common eye-stalk, which in turn communicates with the ventricle of the brain (plate 3, fig. 2). The tapetum nigrum covers the optic vesicles and crosses the midline on the dorsal side of the eyes—that is, between the eyes and the common cerebral vesicle. The tapetum stops abruptly at the optic stem and passes down slightly along the anterior wall which joins the two retinas. The choroidal fissures reach clear through the front of the eyes, running almost together as they approach the common eye-stem, as shown in plate 1, figure 1. The topography of the optic stem, ganglion layer of the retina, and tapetum is in the order given, starting from the midline, in the normal embryo, as is probably the relation of their primordia in the normal neural plate, judging by the work of Eycleshymer and of Lewis. Eycleshymer showed that very early in development the eye appears, in amphibia, as two pigmented spots lying quite close to the midline in the anterior end of the medullary plate. If these groups of pigmented cells are destined to form the tapetum, then the ganglion layer of the retinas would form nearer the midline, while the cells which cross the midline would probably form the optic stem. That Eycleshymer's view is correct is indicated by the work of Locy in his studies upon the shark and by Keibel in his studies upon the pig. According to these authors, the eye primordia arise from small depressions near the midline of the

anterior end of the medullary plate. According to Lewis, certain groups of cells in the medullary plate are predetermined to form the tapetum, the retina, and the optic stem. Lewis's theory has been objected to by Bell, but it has been amply confirmed by Spemann. At any rate, the arrangement of the structures in our cyclopean embryo indicates that the optic stems have been cut out and that the primordia of the retina and tapetum of the two sides have united, blending absolutely with each other across the midventral line.

The tissues of the midbrain and most of those of the interbrain appear to be normal, but our knowledge of the normal brain at this period of development is so scanty that it is dangerous to make any definite statement. Single groups of cells may be wanting or may be blending without our noticing the change. Such a blending is clear only when it involves a sharply circumscribed structure like the eye. However, the tissues of the hypothalamus seem to be disarranged (plate 3, fig. 2), and those of the single united cerebral vesicle (plate 3, fig. 5) are certainly dissociated. In the cerebral vesicle the cells form a uniform layer, which is not beautifully stratified, as is the case in normal development. Over the most anterior part of the brain (plate 3, fig. 5), crossing the midline, is a crescent-shaped cap covering the outside of the brain and reaching back to the hypothalamus just below the point of attachment of the common optic stem (plate 3, fig. 3). This cap is composed of pale cells of uniform size, undoubtedly belonging to the neural tube. It is located on the part of the brain which gives rise to the olfactory lobes in normal development; and it may represent these lobes in a degenerated form.

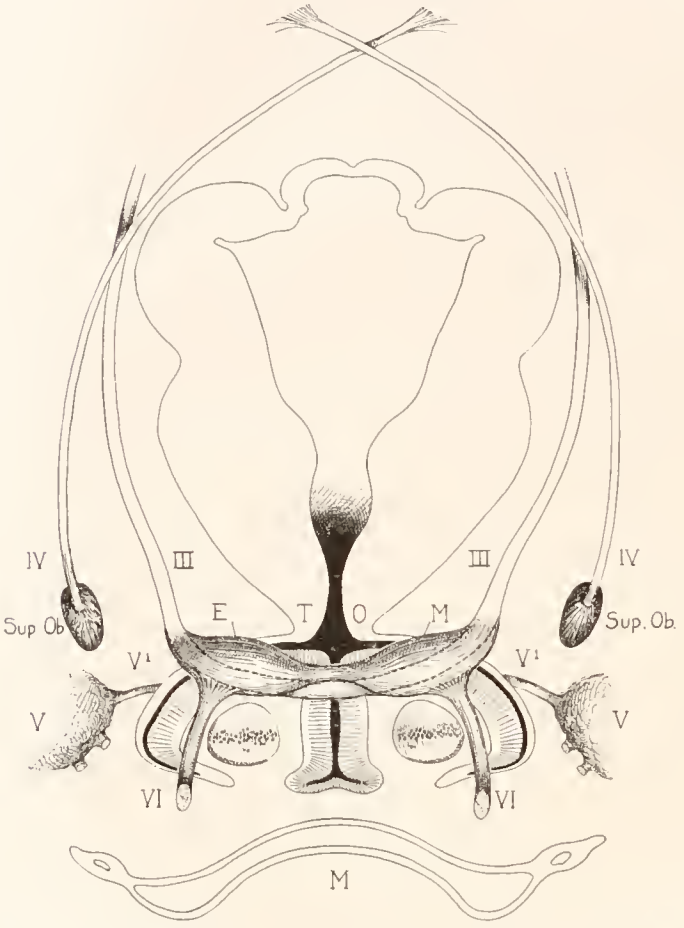


FIG. 1.—Semi-diagrammatic section through the interbrain and cyclopean eye of embryo No. 559. ×50. The cranial nerves are marked with Roman numerals. *Sup. Ob.*, superior oblique; *E–M.*, eye-muscle; *M*, mouth.

It was necessary to make a model (enlarged 100 diameters) of the eye region in order to study carefully the anatomy of the structures of the orbit. In this model the nerves, from the third to the seventh, were worked out to their terminations. The muscle masses, as far as they could be determined, are also included in this model.

The first branch of the fifth nerve is much thinner than the second and third, and passes directly from the Gasserian ganglion back of the eye on either side of the optic stalk; the branches anastomose with each other through several delicate filaments back of the eyes, and then a larger bundle and several very small ones enter the snout, to be lost there. The nerves are shown in section in the figures on plate 3 and in reconstruction in plate 1 and figure 1.

The fourth nerve takes its usual route and finally ends in a very large premuscle mass lateral to the eye and to the first branch of the fifth nerve. It may be noted that this arrangement of the fourth and first branches of the fifth appears to be the reverse of the normal distribution according to Lewis's reconstruction of our embryo No. 163 (fig. 368 of the Manual). In the Lewis reconstruction all of the muscle of the primordium of the eye is blended into a single muscle mass, while in the cyclopean embryo the premuscle mass of the superior oblique muscle is entirely separated from the remaining premuscle mass of the orbit.

The sixth nerve takes its usual course and ends independently in the hour-glass-shaped premuscle mass which crosses the midline between the cyclopean eye and the hindbrain (fig. 1). It is interesting to know that the transverse median muscle mass, as well as the median anastomosis of the third nerve, occurs at the point through which the pharynx gives rise to the hypophysis. In this embryo the chorda ends in the pharynx behind this muscle mass and the pontine flexure of the hindbrain. It appears as though, on account of the great amount of kinking, the region of the infundibulum of the interbrain were pushed away from the pharynx, thus making it impossible for the hypophysis to reach it. As a result of this the third nerve and its muscle masses cross the midline. It may be that the curious cytological changes in the muscle masses (plate 3, fig. 1, *Oc.*) indicate that destructive changes are taking place in them, and that these small round nuclei correspond with the Hofbauer cells as described by Essick in his studies of the transitory cavities in the corpus striatum of the human embryo. The primordium of the eye-muscles show some very remarkable cytological changes. As the sixth nerve approaches the muscle mass of the lateral rectus it is at once observed that this muscle falls into two sharply defined groups of cells, namely, a median group which appears to be normal, and a lateral mass of smaller round cells, the nuclei of which stain intensely. The same grouping is present in the muscle mass of the third nerve. Near the midline the cells appear to be normal, and laterally they are again composed of small round cells. The premuscle mass at the end of the fourth nerve—that is, the superior oblique muscle—can be outlined only with difficulty.

The third nerve shows most remarkable changes in this specimen. It passes along its usual course until it reaches the common eye-stem, over which it circles, for the nerves from the two sides anastomose here, without any diminution in size, within the common median primordium of the eye-muscle (fig. 1). This pronounced anastomosis is also found in another cyclopean embryo, No. 201, in our collection, as shown in figure 3.

Wilder pictures and describes a cyclopean pig (Wilder's fig. 1, plate 2) in which the third nerve arises as a pair in the usual way, which unites after passing through the superior orbital fissure. The union is in the neighborhood of the orbital muscles. He also described the orbit of the cyclopean eye in a large double-headed fetus. By dissection of the head he found that the two third nerves, one coming from each brain, unite with each other and form a common trunk stretched transversely across the midline back of the eyeball. From this anastomosis small twigs arise to supply the muscles of the eyes (Wilder's fig. 6, plate 3). In his work on monsters (page 282), Ahlfeld states that Delle Chiaie described a specimen having a similar anomaly, which was published in Naples in 1840. Delle Chiaie gives an excellent illustration of this specimen, with a diagrammatic section of the head, showing the eye and its attachments. This picture is copied by Ahlfeld on plate 46, figure 18. It is somewhat difficult to identify all the structures given, but he apparently pictures the two third nerves anastomosing before they reach the single eye. He also pictures a branch of the fifth nerve passing into the snout, which, as in our specimen, contains a cavity. I have been able to find one more specimen in the literature in which there is an anastomosis of the two third nerves within the orbit in cyclopia. This is in the excellent description of Dr. Black on the nervous system of a cyclopean specimen at birth. Black alludes to the third nerve in a single sentence. He says (on page 204) that in the region of the central tendon the third nerve divides into a number of small branches, each of which communicates with its fellow on the opposite side. I have given these references, as they are the only ones which I can find in the literature, and they invariably accompany the description of the orbit in cyclopia. The anatomy of cyclopia in monsters is rarely given; and it may be remarked that until we have numerous good descriptions (like that of Black) of the eyes, central nerves, and face in cyclopia, we shall not understand fully the anatomy of this most interesting type of specimen.

In a *Janus* monster (No. 1178, *a* and *b*) at birth with cyclopia on one side, Dr. Theodora Finney has demonstrated a large anastomosis behind the orbital branches of the third nerve before they are distributed to the eye-muscles. An account of her specimen with a figure is given on page 30.

It remains to attempt to correlate what has been said above regarding cyclopia with the form of the brain and the optic vesicle in early human embryos. Before undertaking this attention is called to two papers by Tandler on the form of the early brain in *Tarsius* and in *Platydactylus*. Tandler's papers are especially noteworthy for the reason that the topography of the forebrain has been determined with a greater precision than has been carried out in the human embryo, except possibly in the most recent work of His. There is sufficient material at hand to make similar studies upon the human brain, but until this is done I must content myself with what has already been published, alluding occasionally to several of the specimens in our own collection.

A specimen 3.2 mm. long, with 13 or 14 myotomes, was described with much care by His in his last publication upon the brain. He has illustrated this specimen by a sagittal median section through the body as well as by the external and

internal forms of the brain. These illustrations are given in the His monograph as figures 2 and 33, and they are also copied by Streeter in his article on the brain in the Manual. It may be stated that figure 33 shows the neuropore nearly closed, the optic stalk being still represented as a wide-open canal which reaches to the midventral line. Dorsal to the optic stem is a slightly marked pocket which reaches to the neuropore, and it is believed by His that this represents the beginning of the cerebral hemisphere. It is interesting to note that Keibel and Else state in their Normal Plates (page 100) that the cerebral vesicles are just beginning in embryos 4.5 to 5 mm. long. It may be that His exaggerates this pocket slightly in his model, but it is of great value to us to have his opinion regarding the location of the primordia of the cerebral hemispheres in the brain-tube before the neuropore is closed. According to Watt, the cerebral hemispheres arise much more dorsal than is indicated by His in his figures.

Keibel and Else give excellent illustrations of the form of the brain up to the time of the closure of the neural tube. No doubt the Kroemer-Pfannenstiel embryo, which contains five or six myotomes, represents a normal stage with medullary plates wide open, and in this embryo there is no indication whatever of an eye primordium. The same is true in the Kollmann embryo, containing 14 myotomes, which is illustrated by Keibel and Else as figure 4. This specimen also seems to me to represent a normal embryo, as we have in our collection a stage that is practically identical with it. Our embryo No. 391 contains seven pairs of somites, and has been carefully studied and published by Dandy. A model of it, as well as sections through the head, has also been pictured by Evans in figures 408 and 409, Volume II, of the Manual, and it shows no out-pocketings in the anterior part of the brain to represent the eye primordia. However, when we reach Keibel and Else's figure 5, which is taken from the Pfannenstiel III embryo, two marked diverticula are seen to arise from the front end of the neural tube, which Keibel, in his article on the eye in the Manual of Embryology, believes to represent the primordia of the eye.

As Keibel and Else have reproduced numerous figures of sections through the head of this embryo, it is easy to ascertain the exact form of its neural tube. However, I am of the opinion that we can hardly view the neural tube in this embryo as normal, as it is not sufficiently advanced in development for an embryo of this stage and as it corresponds very much in form with the brain of our embryo No. 12, which I believe to be pathological. At this time the neural tube should be nearly closed, while in Keibel and Else's specimen and in our No. 12 it is still wide open. The Pfannenstiel III embryo contains the same number of somites—that is, 14—as our embryo No. 12, and the form of the brain and of the eye-vesicles is very similar in both embryos. A picture of the external form of embryo No. 12 will be found in my article on the development of the intestines (plate 19, fig. 2). My reason for believing that the brain form in both of these embryos can not be viewed as normal is that in other young specimens published recently by Wallin and by Bremer quite a different form of the brain-tube is shown for this stage of development. The Wallin specimen, which contains 13 somites, has the brain-

tube pretty well closed up, leaving a large neuropore in the front. The Bremer specimen is slightly in advance of this. We have also in our collection a similar embryo (No. 470) in which the brain form corresponds exactly with that in the specimens of Wallin and of Bremer.

Furthermore, the twin specimens of Watt, which contain 17 or 18 somites, correspond very closely with the three above-named embryos. In the Watt specimen the neuropore is nearly closed and the eye-vesicles reach all the way across the front of the brain-tube. The Pfannenstiel III embryo and the Bremer embryo are found pictured by Bach and Seefelder, both in profile and in sections, but I do not think we should unreservedly accept their description of the beginning of the eye-vesicles as final for the human embryo.

It seems to me that our knowledge of the form of the forebrain before closure of the neuropore is much in need of revision, and towards this revision we have assembled several new models of young embryos. The first is a model by Dr. Bartelmez of our No. 1201 (University of Chicago, No. 87) from an embryo with 8 pairs of somites, which seems to me to bear very much upon this question. The neural plate flanges out into a large tongue with a slightly hourglass-shaped depression running across the midventral line (plate 2, fig. 6.) The larger lateral depressions no doubt indicate the foveola, and the groove connecting them across the midline is in the position in which the optic stalk develops later on. It would probably be better if we accepted Froriep's designation of torus opticus for this connection. The torus opticus seems very insignificant in this specimen, as shown in the illustration (*t. o.*), but when we consider to what extent the torus may be stretched, as illustrated by Bach and Seefelder (fig. 2, plate 13), we recognize the importance of this structure. I am inclined to believe that the form of the brain in the Bartelmez embryo must be viewed as normal, as it corresponds so well in a series with that of several older human embryos, namely, those recently described by Wallin and by Bremer and our No. 470.

There is another ridge already indicated in the brain-tube of His's specimen EB reaching across the midline just below the neuropore. This is the torus transversus of Kupffer; and those who are interested in this structure are referred for greater details to the articles by Tandler, Kupffer, and Johnston. The neuropore is found just closed in our embryos No. 148, published by Mrs. Gage, and No. 836, which has been modeled by Dr. Evans, as well as a specimen of the same size modeled by Johnston. Dr. Johnston has been good enough to send me photographs of his model, so that I am able to compare it with our own. It is clear to me that the large "optic vesicle," extending over the whole lateral wall of the front part of the neural tube, represents more than the optic vesicle, as it must also include the primordium of the cerebral hemispheres, since the torus transversus touches the lower border of the neuropore and the optic vesicles fall below this line. This large so-called optic vesicle must resolve itself into the optic vesicle and brain hemisphere in subsequent development. In this process the torus opticus gradually must become more pronounced.

It is somewhat easy to compare the medullary plate of the Bartelmez embryo No. 1201 with the medullary plate in amphibia. The lateral foveola corresponds with Eycleshymer's pigmented spots, and if these areas were cut out eyeless embryos should be produced, as Stockard found in his experiments on *Amblystoma*. If it is admitted that the eye primordia communicate across the midline through the torus opticus, then Stockard's experiments upon the medullary plate may be interpreted as follows:

No important organ develops from the midline of the medullary plate, and this is represented only by a thin layer of cells. It has been called by His the floor-plate. The motor nuclei arise from the thickenings on either side of the floor-plate, and these are known as the basal plates. The narrow, thin floor-plate really forms the ventral midseptum of the spinal cord of the brain, and subsequently commissural fibers grow through it to form the raphe. If we view the basal plate from above, as shown in embryo No. 1201 (plate 2, fig. 6), we find that this raphe should extend forward to the neuropore, at which point the raphe fibers are the anterior commissure within the torus transversus. Back of this we have the torus opticus, and its commissural fibers are the fibers of the optic nerve. At an early period in its development the torus opticus must widen rapidly and push through the rest of the brain, for the optic stalk appears quite suddenly, and an injury to the medullary plate at this time would probably make itself felt more upon the optic stalk than upon the eye, for the short period in which it grows very rapidly is its critical period. If we cut out the optic stalk or the torus opticus, as Stockard and Lewis did in their experiments, then the foveola would remain together and form cyclopia. Stockard and Lewis also note that in their experiments they frequently obtained embryos with but one eye which appeared to be quite normal. This is to be expected when experiments are made upon two primordia which lie very close together; and when either the left or right eye primordium is removed, left or right-eyed embryos are produced, but not cyclopia. Lewis had to destroy only the midline of the embryonic shield in order to produce true cyclopia. It may be added that the anatomical changes found in our small cyclopean human embryo, as well as in all cyclopean human monsters, can be explained by removal of the structures represented along the line of the raphe of the medullary plate reaching from the mammillary bodies to the neuropore. This includes the torus transversus, which naturally involves the olfactory region and the anterior commissure. Thus we can explain by a study of this specimen the anatomical changes of the brain found in human cyclopia.

### Stunted Embryo, GL 20, with Cyclopia, Carnegie Collection, No. 201.

The second specimen of cyclopia in our collection is a pathological embryo, which is not very well preserved, measuring GL 20 mm. This specimen came from Dr. Schelly of Baltimore, who obtained it from an abortion on February 7, 1902. He brought it to Mr. Brödel, and subsequently, because it was pathological, it was given to me. The embryo was incased in an ovum covered with a ragged decidua, together measuring 80 by 60 by 50 mm. Upon opening this

ovum it was found filled with a jelly-like fluid forming a type of magma described in my paper upon this subject. Sections were cut of the fleshy chorion and it was found that the wall is composed of a true chorionic membrane, villi, maternal blood, fibrin, decidua, blood sinuses, and trophoblast, with an extensive infiltration of leucocytes often accumulating in large masses or small abscesses. These layers are not in any regular order, but are intermingled, and show various stages of disintegration. The mesenchyme of the villi is fibrous, and many of these are invaded by trophoblast cells as well as by leucocytes. The trophoblast also invades the blood-clot, and maternal-blood sinuses are frequently found filled either with trophoblast cells or with leucocytes. There are certain groups of tissue in which the intermingling of the trophoblast cells within the fibrinoid substance appears, in sections, very much like cartilage. Most of the decidua adjacent to active trophoblast on the tips of some of the villi has the usual fibrinoid layer characteristic of normal development. It may be added that the structures of the chorionic membrane, as well as those of the embryo, stain unusually well, which indicates that the tissues were active and alive at the time of the abortion.

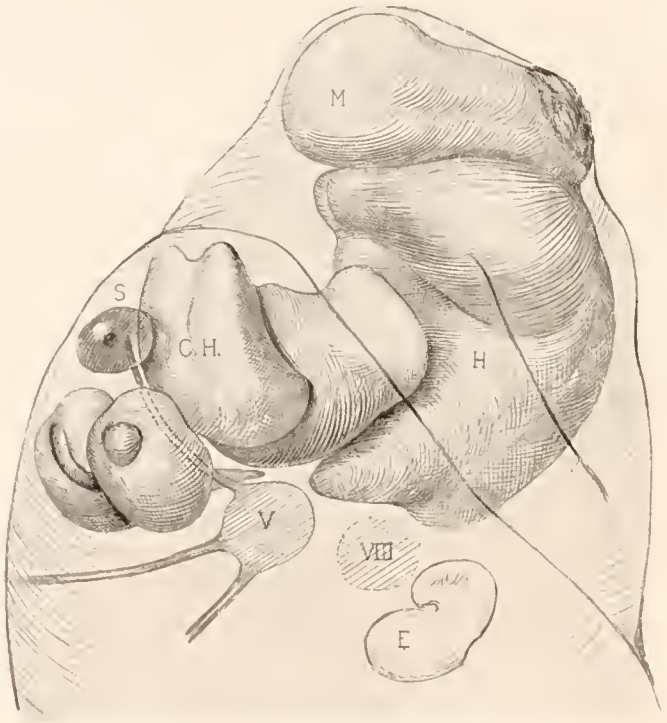


FIG. 2.—Reconstruction of the head of embryo No. 201. ×25. The outline of the head was obtained from a photograph, the brain and eyes from a plaster reconstruction, and the nerves were added from the sections. *C. H.*, cerebral hemisphere; *M*, midbrain; *H*, hindbrain; *S*, rudimentary snout; *V*, fifth nerve; *VIII*, eighth nerve; *E*, external ear.

After the embryo was received it was photographed from one side (plate 2, fig. 1), and this record proved to be very useful in making several reconstructions. The embryo was then cut into serial sections 50 mm. thick; finally, in order to study more carefully the structures within the head, a reconstruction of its external form, as well as of the form of the brain and the eye, was made in plaster of paris at a scale of 40 diameters.

Most extensive changes have taken place within the embryo. The brain is greatly deformed and is severed from the spinal cord through a growth of tissue in the region of the medulla back of the deformed ear. In fact, a part of the brain is included within the cap-like body on top of the head. The spinal cord begins again quite abruptly in the upper cervical region and ends with the same abruptness in the upper lumbar region. At its lower end there is a curious fibrous tumor measuring half the diameter of the cord. The cord, so far as it is developed,

appears to be normal, but it is markedly dissociated. Below the upper lumbar region the spinal cord is wholly wanting and the spinal canal is filled with mesodermal tissue which is rich in blood-vessels. Where the cord is missing most of the spinal nerves still remain, and many dorsal ganglia can be made out, showing that the changes in the central nervous system in this region took place after the spinal nerves had been developed from it.

Most of the epidermis is intact, but it is broken through at the back of the head, where there is found an extensive ulcer or necrotic mass, which is very rich in blood-vessels and involves the walls of the brain but does not reach into its ventricle. At the highest point of the head the epidermis has developed into a papilliform body, and below this there is a large necrotic area in which there is a great quantity of yellow pigment granules.

The mouth is closed completely, although the alimentary canal, from the mouth to the stomach, is open and appears normal. The intestine is matted together; the cloaca and anus are obliterated. The epithelium of the upper portion of the intestine gives rise to marked growths into the matted mass.

The thoracic region, liver, and vascular system have undergone practically no change. The extensive dissociation of the tissues throughout the embryo has caused an extensive destruction and arrest in the development of the muscular system. This is marked by all kinds of secondary changes in the connective tissue, especially in that of the skin, where the change is pronounced, as may be seen in plate 2, figure 4. In this region the change is so great that it obliterates entirely the external auditory canal.

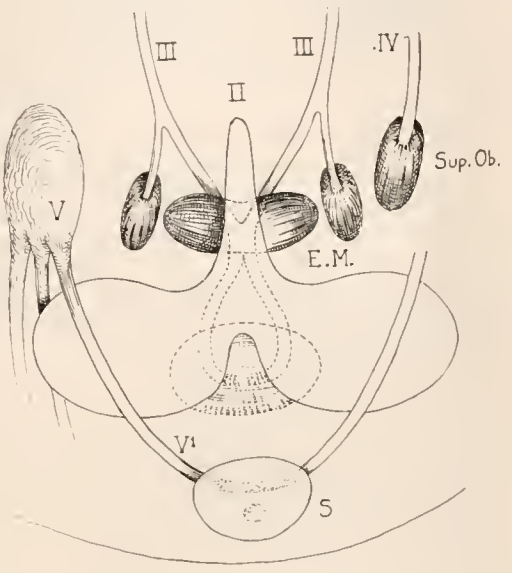


FIG. 3.—Diagrammatic reconstruction of the eye and the surrounding structures of embryo No. 201. ×25. The cranial nerves are marked with Roman numerals. $V^1$, first branch of fifth nerve; *S*, rudimentary snout; *E. M.*, eye-muscle; *Sup. Ob.*, superior oblique muscle.

A reconstruction of the brain and upper part of the spinal canal enabled me to determine with greater precision the parts of the brain which are within the head of this embryo. Subsequently it was possible to find the remnants of the ganglion of the eighth nerve; then that of the fifth nerve was determined. Their position is shown in the profile diagram of the outlines of the brain and these nerves (fig. 2). It at once becomes apparent that the portion of the brain extending up in the necrotic cap on the top of the head consists of the midbrain and hindbrain. With this idea in mind it was possible to answer the question in the serial sections, as the walls of the midbrain are thick and the ventricle relatively small, and the walls of the hindbrain are thin and its ventricle relative large. The pointed process which reaches towards the ganglion of the fifth therefore represents the pontine flexure of the medulla. It is now easy to interpret the

isolated brain-mass between the eye and the large mass just described. By comparing these structures with a profile reconstruction of the forebrain of the cyclopean embryo No. 559 (plate 1, fig. 1), the larger mass just above the ganglion of the fifth undoubtedly represents the interbrain, as the free end of the optic nerve reaches just to its base but does not enter it. The crescent-shaped mass of the brain in front of the interbrain is the unpaired single cerebral vesicle, which communicates freely with the ventricle of the interbrain. The structure of the wall of this degenerated cerebral vesicle corresponds very closely with that of the small vesicle in embryo No. 559. With these structures established, as shown in the profile reconstruction, it is possible to identify some of the remaining structures of the orbit. However, the tissues are very greatly dissociated, and for the present it is impossible for me to follow them farther than is given in the following description. First of all, the three branches of the fifth nerve can be followed to their termination. The first branch reaches up to the rudimentary snout, where it anastomoses with its fellow from the opposite side—shown also in the diagram (fig. 3). This snout, by the way, is represented by a slight elevation in the middle of the face above the eye, and sections through it show that it is composed of a relatively large, irregular mass of cells which are separated more or less from the surrounding mesenchyme. Within the middle of this mass is a small pearl-like body about 0.1 mm. in diameter. Just behind this body the first branch of the fifth nerve anastomoses across the midline.

FIG. 4.—Section through the cyclopean eye of embryo No. 201. ×40. $V^1$, first branch of fifth nerve; *S*, snout.

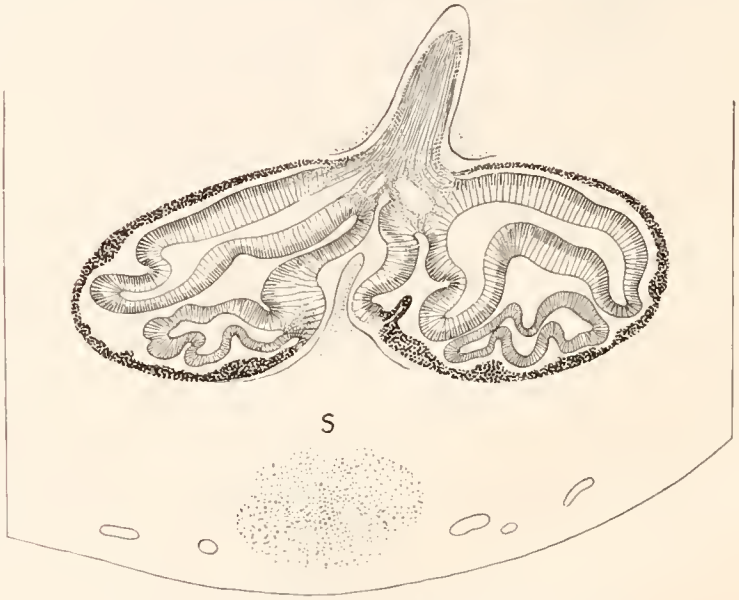


FIG. 5.—Section through the cyclopean eye and optic nerve of embryo No. 201. ×40.

The second branch of the fifth runs below the eye on either side and reaches nearly to the skin, where it spreads out into several large branches. The third

branch of the fifth nerve runs deep into the neck and is separated from the second by a pronounced ossification center representing, of course, the maxillary bone.

The two eyes are united, forming an hourglass-shaped body with a double retina, a single pigmented layer, and a single optic nerve which arises from the retinas as they approach each other (figs. 4 and 5). The tapetum is continuous over the superior surface of the eyes, but it is broken below, repeating the condition found in the eye of embryo No. 559. The optic nerve reaches to the base of the interbrain, where it ends abruptly. It is impossible to determine with precision the arrangement of the muscle masses of the orbit or of the nerves passing to them. That no trace of the sixth nerve could be made out is shown in figure 3, and this may be accounted for by the fact that the organ which gives rise to the sixth nerve has undergone extensive degeneration. However, the peripheral ends of the third and fourth nerves can be found, but they can not be traced back very far in the direction of their origin. The fourth nerve is thicker than normal and ends on the lateral side in an enlargement which may represent the superior oblique muscle. Below the optic nerve is a common muscle mass which crosses the midline, and to either side of this there are two independent muscle masses. Before the third nerve reaches these muscle masses lateral branches are given off, which pass to the second lateral muscle mass, as shown in the diagram. The two nerves then approach each other and communicate freely through the unpaired muscle mass, and then pass forward under the cyclopean eye and finally end just beneath the skin.

A comparison of plate 1, figure 1, and text-figure 1 with text-figure 3 shows that the first branch of the fifth nerve in both embryos anastomoses across the midventral line in the region of the snout and that the two third nerves anastomose with each other through the main muscle primordium of the orbit. Both of these anastomoses must be viewed as secondary, for the two nerves must have been single when they arose from the brain. This observation favors the theory that the eye promordia must also have been bilateral—that is, they must have been separated by a narrow strip of non-ocular tissue in the normal medullary plate.

### Fetus Compressus with Cyclopia, Carnegie Collection No. 1165.

This embryo was sent to me by Dr. Ralph S. Perkins, of Exeter, New Hampshire. Only the embryo was received, which measures 43 mm. CR. It was found to be greatly distorted; the umbilical cord is of thread-like thinness, and the development of the different parts of the body seems to be unequal. Apparently some of the joints are dislocated, but at present it is impossible to say whether or not these distortions are due to mechanical manipulation after the embryo was aborted. This is possible, because the embryo had been wrapped in a towel some time before it was fixed in formalin, but a careful study of the sections demonstrates that the specimen is quite a typical fetus compressus.

The embryo came from a white woman, 35 years old. Her first child is 16 years of age; the second died at the age of 2, and the third is 12 years old. These are all by her first marriage. The first pregnancy of her second marriage

ended in an abortion at 5 months, and then she gave birth to a child which is now 21 months old. The next pregnancy resulted in an abortion at 5 months, and the last one gave the specimen under consideration.

Her last normal menstrual period began on February 25, 1915. The next period began on March 21 and continued for only one day; and this was followed by the abortion on May 2. There are no other data bearing upon this case except that 15 years ago the woman had an operation for suspension of the uterus.

Upon careful inspection of the head of the specimen a mechanical injury just below the lower jaw was found, as shown in figure 6. The ear seems to be distorted or abnormal, and in place of the nose and eyes there is a depression in front of the face, and running from it is a cleft reaching to the mouth. Apparently we have here a fetus compressus with cyclopia and hare-lip.

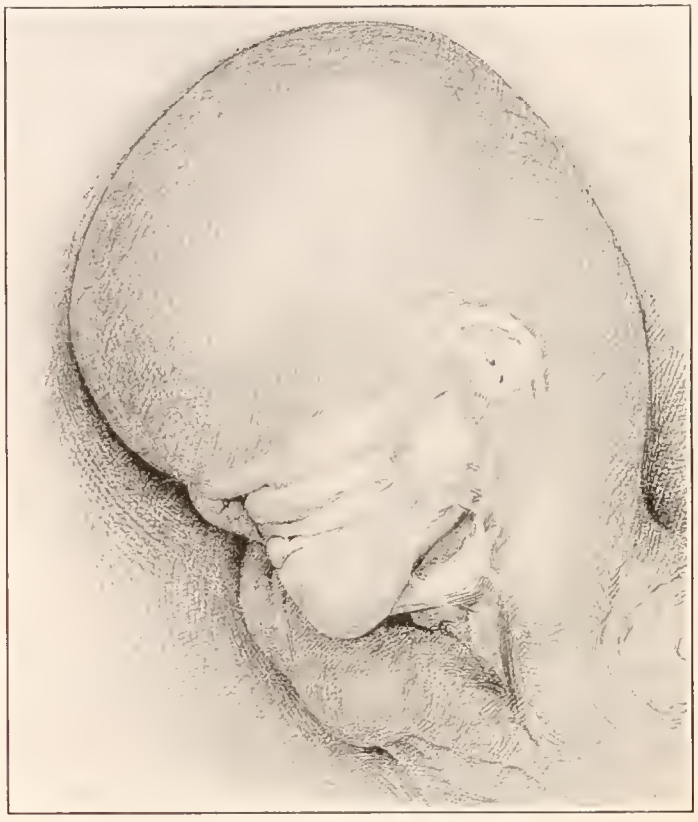

FIG. 6.—Direct drawing of the head of embryo No. 1165. ×4.5. The tissue of the lower jaw is injured. The depression from the cyclopean eye extends down into the mouth, forming hare-lip.

The head of the embryo was stained *in toto* in cochineal and embedded in paraffin. It was cut into serial sections 50 $\mu$ thick. The sections show that all the tissues are markedly dissociated, and in addition the brain is completely macerated. In fact, the brain-cavity appears like a bag filled with débris, which reaches down into the cervical region of the neck and terminates abruptly where the spinal canal is filled with a new formation of fibrous tissue. The primordial skull is composed of cartilages which have undergone some fibrous changes, and their borders are not sharply defined, but grade over into the surrounding connective tissue. The cartilages at the base of the skull appear to be enlarged and extended; but this point can not be established without making an elaborate reconstruction. In the cervical region the bodies of the vertebræ are displaced backward into the spinal canal, which in turn is largely filled up with the newly formed fibrous tissue as well as with numerous round cells. The tissues of the various ossification centers have undergone a curious change, reminding one of necrosis. It appears as though the ossification centers had died while the surrounding cartilage had continued growing. It is difficult to define precisely the muscles and nerves in all of the various sections, while at points certain muscle groups seem to retain their normal form.

Most of the epidermis is wanting and in the region of the face are large skin protuberances composed principally of round cells. Such protuberances form the lids of the cyclopean eye, as shown in plate 2, figure 3. The orbital cavity lies upon the cribriform primordia of the maxillary bones and is filled with a single group of pigmented cells, which is surrounded by an infiltration of round cells. Back of this pigmented mass are the primordia of the eye-muscles, but their dissociation is so complete that it is impossible to locate the individual muscles, nor can any of the nerves be made out with precision. Aside from the pigmented mass there are no remnants of the layers of the retina, these having undergone complete dissociation. In the upper part of the orbital mass is a curious gland-like structure badly dissociated, which may represent the lacrymal gland. We have, therefore, in this specimen the remnant of a single median eye represented by an irregular but rounded mass of the tapetum situated below the depression of the skin. In turn this depression is partly covered with folds of dissociated tissue which may be recognized as the eyelids of the cyclopean eye.

### Cephalothoracopagus Monosymmetros with Cyclopia on one side, Carnegie Collection No. 1178 *a* and *b*.

The double female monster, 205 CR and 350 GL long, weighing 1,624 grams was sent to us by Dr. J. I. Butler, Rodgers Hospital, Tucson, Arizona, on May 14, 1915. The mother is a Chinese woman, age 24, who has given birth to three children at term and has had two abortions. Apparently the uterus is normal and there is no history of venereal diseases. There is nothing else in the history that bears upon this case. The specimen has been completely dissected by Dr. Theodora Finney, who has given me the notes for the following description of the muscles of the orbit and the nerves of the cyclopean eye. A more detailed account of the anatomy of this interesting specimen will be published by Dr. Finney at some subsequent date.

The fetus is composed of two nearly complete bodies which lie with their anterior surfaces toward each other, and, as the name implies, are fused from the umbilicus up, forming one thoracic trunk and one head. There are two independent spinal columns, eight extremities, and two composite fronts, every symmetrical part of which is formed half of one and half of the other individual. There are also two faces, one of which is well formed, while the other is a synote with a cyclopean eye and snout situated above it. In dealing with the cyclops, then, it must be noted that its left half is formed from the left side of one individual, while its right half is from the right side of the other individual.

Internally the thoracic and abdominal viscera are double, with the exception of the esophagus, the stomach, and the upper part of the intestine, which are united with a single canal. There are two central nervous systems, separate and complete to above the level of the two hypophysi, where fusion occurs. As much tissue was lost from the region of the thalami in removing the brain, the mode of union of the base of the brain could not be determined. An optic chiasm, however, belonging to the well-formed face, remains *in situ* immediately behind the

orbits. This shows there is a true normal union for the two individuals at this point. In the cranial cavity behind the cyclopean eye one optic nerve-stalk, composed of two bundles pressed together, is observed.

The dome of the cranium is filled by three cerebral bodies; two of these are recognizable hemispheres, though much shortened antero-posteriorly. Their position is normal, behind the well-formed face. They possess well-defined but shallow cortical sulci. The third cerebral division consists of a kidney-shaped lobe. Its cortex is smooth, except for two or three atypical creases near the poles. It lies transversely across the cyclopean side of the cranial cavity with its two poles directed inferiorly, the convex portion between them straddling the single orbit. It represents fused cerebral tissue obtained from both individuals.

The cyclops has a well-formed eyeball to which four pairs of muscles are attached; their arrangement is shown in figure 7. These muscles can be identified by their nerve-supply as being the muscles of the upper and outer parts of the two fused eyes. These muscles are changed from their normal positions, so that they entirely surround the eye. The muscles are the superior obliques, the levator palpebræ, the superior and lateral recti. The two superior obliques lie near to the midline on the superior surface. Slightly lateral to these, though still on the superior surface, lie the two levator palpebræ. On the sides, in the place usually occupied by the lateral recti, lie the superior recti, which are shifted downward from their normal position through an arc of 90°. About the same amount of shifting causes the lateral recti to lie close together on the inferior surface of the eyeball. The inferior oblique muscles and the medial recti are completely eliminated. The inferior recti are entirely absent at the bulbar end. There is a short bundle of muscles underneath the proximal end of the lateral recti, which probably represents remains of the inferior recti. The lacrymal glands have participated in the change of position and fusion. Their tissues lie as a broad single gland-mass on the inferior surface of the bulb.

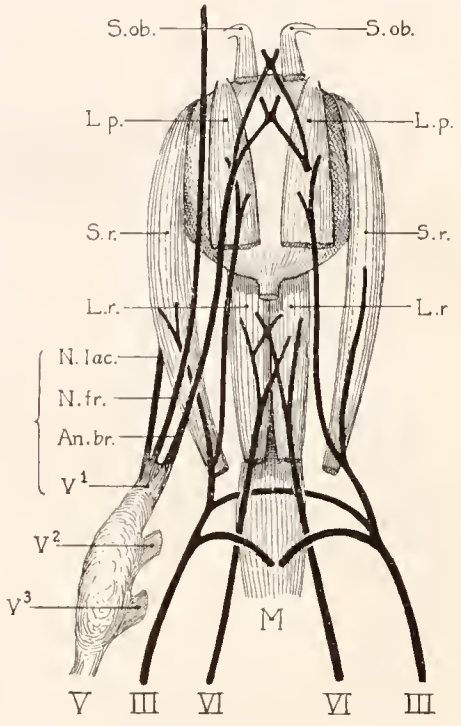


FIG. 7.—Diagram of cyclopean eye and its appendages of the *Janus* monster, No. 1178 *a* and *b*, from a sketch and dissection by Dr. Finney. For the sake of clearness the superior oblique muscles are moved forward. The cranial nerves are marked with Roman numerals. *S. ob.*, superior oblique muscles; *L. p.*, levator palpebræ; *S. r.*, superior rectus; *L. r.*, lateral rectus; *M*, rudimentary muscle-mass, probably the remains of the inferior recti. It is noticed that the first branch of the fifth nerve gives rise to a trunk which anastamoses across the midline. The same is true of the third and sixth nerves.

In order to be sure that the identification of the nerves passing to the muscles of the cyclopean eye was made correctly, they were carefully compared with the normal nerves of the eyes of the well-developed face. This comparison left no doubt as to which nerves were being traced to the single eye, as the points of origin

from the brain-stem of the third, fourth, fifth, and sixth nerves were symmetrical for both faces. The arrangement of the nerves on the cyclopean side are as follows:

1. The olfactory nerves are absent.

2. The origin of the optic nerve was lost. Two small and flattened optic nerves, however, pass out together in the dura. These finally fuse into one stalk which ends in the bulb. This stalk, 2 mm. in diameter, is about the same size as the normal optic nerves of the well-formed face on the opposite side.

3. The two third nerves which belong to the cyclops are 0.5 mm. in diameter at their point of origin and throughout their course, while the third nerves on the opposite side which pass to the perfect face are twice that size. The cyclopean oculo-motor nerves pass into the dura, where they run toward each other to the place where the eye-muscles arise. Here these nerves lie within 3 mm. of each other. Branching occurs in this region. Two of these branches fuse immediately. There are two other pairs of main branches which innervate the levator palpebræ and the superior recti on each side of the single eye. There are some finer branches whose course could not be definitely ascertained.

4. The cyclopean fourth nerves are equal in size with those of the normal eye. They run as two fine threads to within a few millimeters of each other, when they turn anteriorly and run parallel on the surface of the superior oblique muscles, in which they terminate.

5. The two Gasserian ganglia of the cyclops are somewhat smaller than those of the normal face. Each has three divisions: ophthalmic, maxillary, and mandibular. The two ophthalmic divisions have each three main branches. One of these branches passes along the roof of the orbit and makes several χ-shaped anastamoses with its fellow near the front of the eyeball. Another runs forward, parallel with its fellow, out into the skin, where they are both cut; so if anastamosis occurred it could not be determined. The third and last branch, one on each side of the eye, ends in the lacrymal gland.

6. The sixth nerves of the cyclops, about 0.8 mm. in diameter, are equal in size with the sixth nerves, passing to the well-developed face. They converge to the base of the orbit when they run parallel to each other on the upper side of the lateral recti muscles, in whose substance they terminate after making several χ-shaped anastamoses.

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## EXPLANATION OF PLATES.

### Plate 1.

1. Plaster reconstruction of the brain and cyclopean eye of embryo No. 559. × 25. Cranial nerves are marked with Roman numerals. *o. v.*, optic vesicle; *s*, snout; *m*, mouth.
2. View of the right side of embryo No. 559. × 9. Only the face region is worked out in detail. *U. v.*, umbilical vesicle.
3. Face of embryo No. 559. × 16. The drawing is made from a plaster-of-paris reconstruction. *S*, snout.
4. View of the left side of cyclopean embryo No. 559. × 9. The drawing was made directly from the specimen in formalin.

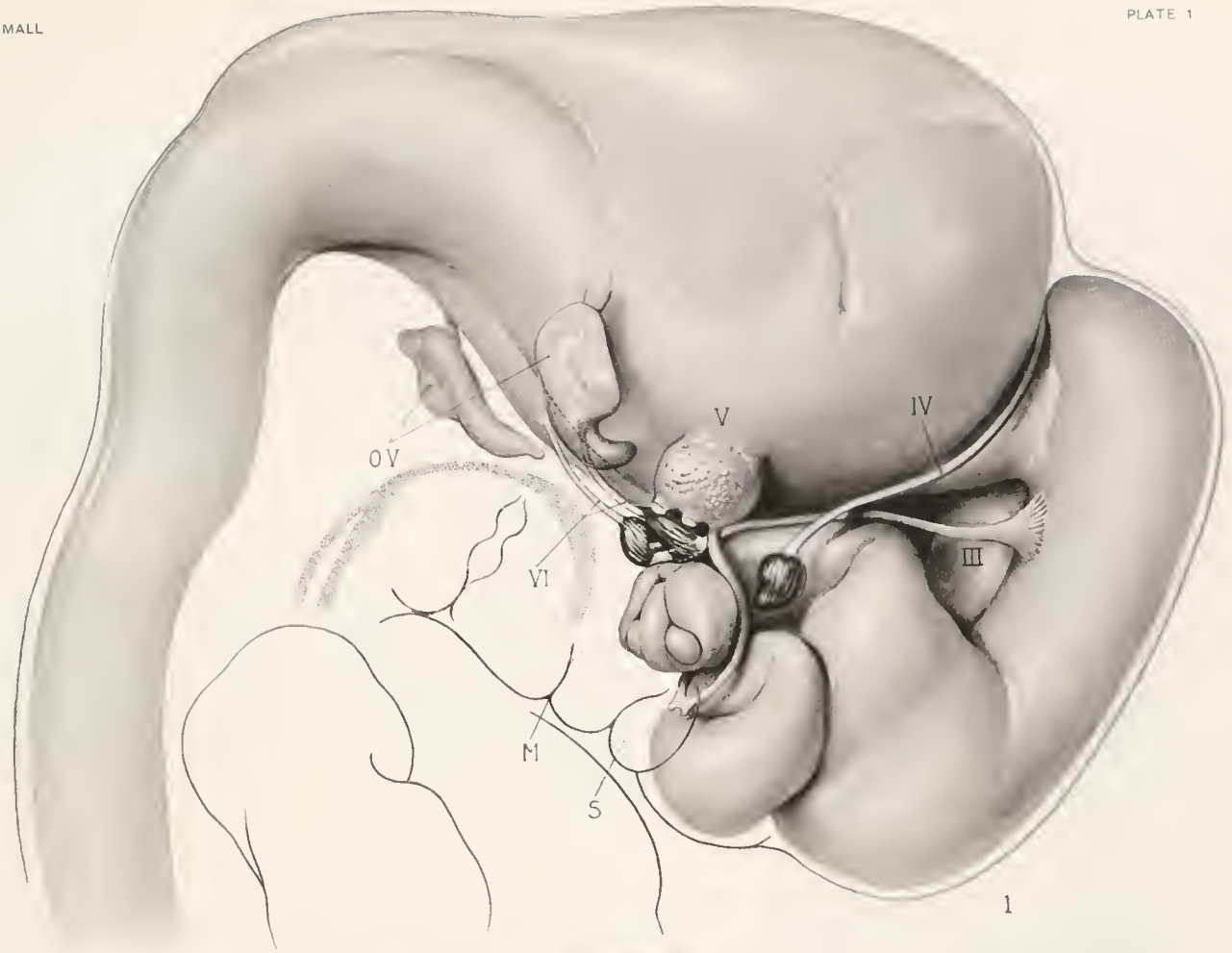
### Plate 2.

1. From the photograph of embryo No. 201. × 1½.
2. Photograph of ovum, No. 559. × 1.
3. Section through the cyclopean eye, No. 1165. × 40. *E*, eye; *e. l.*, eyelid. Behind the eye are seen the ocular muscles.
4. Section through the external ear of embryo No. 201. ×75. There is an invagination of the epidermis and tissues of the ear are dissociated.
5. Photograph of the ovum, No. 559, showing the embryo in position. × 3. The exocœlom is filled with a dense magma.
6. Outline of brain of embryo No 1201. × 100. From a plaster reconstruction made by Dr. Bartelmez. The wide-open flange in front contains two depressions, the optic vesicle, which unite through a common groove, the torus opticus, as marked on the drawing. The depression behind this flange no doubt represents the cavity of the midbrain and hindbrain. This specimen is No. 87 of the collection of the University of Chicago and contains eight pairs of myotomes.
7. Photograph of section through the snout of specimen No. 559. × 60. The frontal process contains the common cerebral vesicle, *c. v.*, and below the snout there is the upper jaw, *u. j.*

### Plate 3.

All the photographs are from the sections of the embryo No. 559. Figs. 1 and 2, ×50; figs. 3, 4, 5, ×40.

1. Section through the ocular muscle, showing the terminal fibrils of the third and sixth nerves. *Oc. mus.*, ocular muscle; *n*, notochord; *m*, mouth; *v*, fifth nerve.
2. Section through the eye at its attachment to the interbrain. *T. o.*, torus opticus; *v′*, *v″*, *v‴*, branches of the fifth nerve; *iv*$^{4}$, terminal filaments of the fourth nerve ending in the primordia of the superior oblique muscle.
3. Section through the lower tip of the cerebral hemisphere. The peculiar tissue surrounding the nerve-body may represent a degenerated olfactory region. About this is seen a section of the interbrain, and below a process containing the cyclopean eye. *Fb*, first branch of fifth nerve; *m*, mouth.
4. Section through the middle of the cyclopean eye. *I. b.*, interbrain; *Fb*, first branch of fifth nerve; *m*, mouth.
5. Section through the cerebral hemispheres as they communicate with the interbrain. *I. b.*, interbrain; *c. h.*, cerebral hemisphere; *s*, snout; *u. j.*, upper jaw. Over the lamina terminalis is seen the peculiar thickening of the outside of the body which may represent the degenerated olfactory region also shown in the flat section of figure 3.

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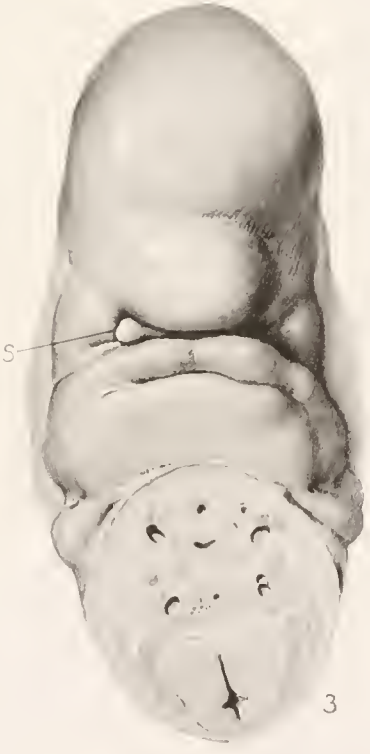


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Fig. 1.

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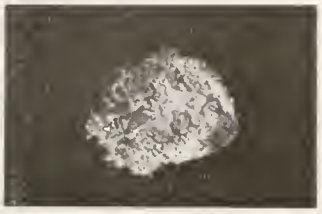

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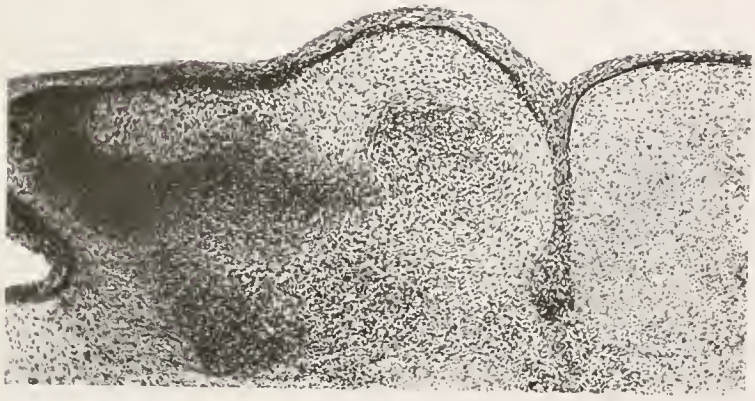

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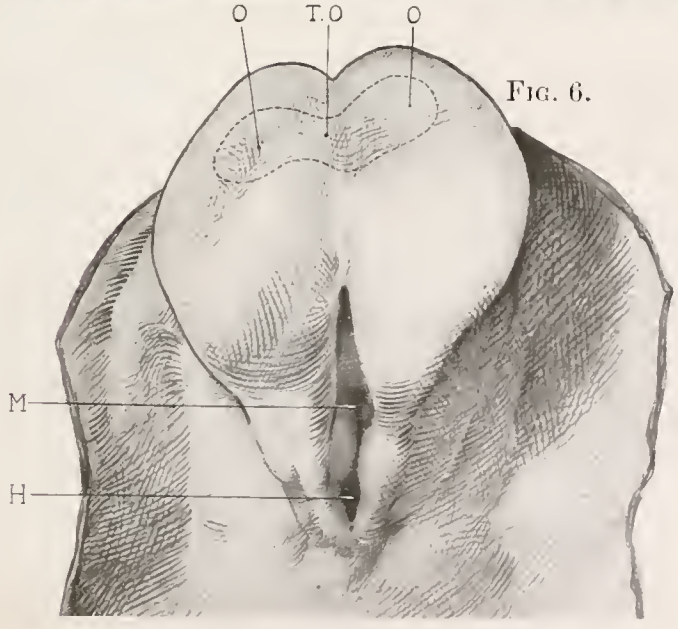


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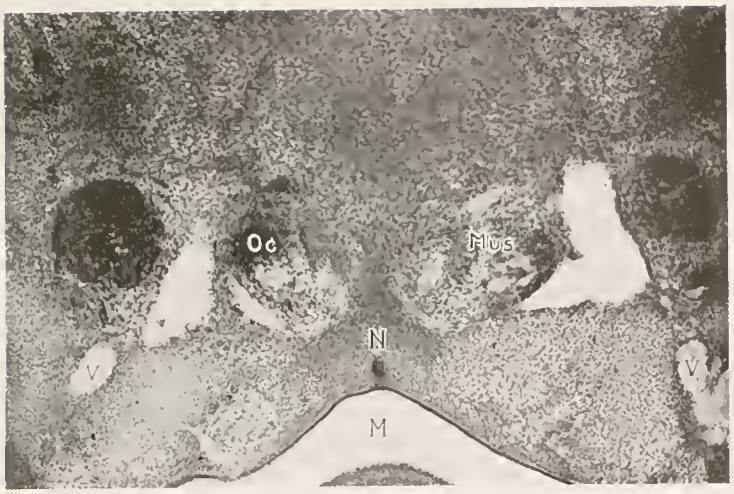
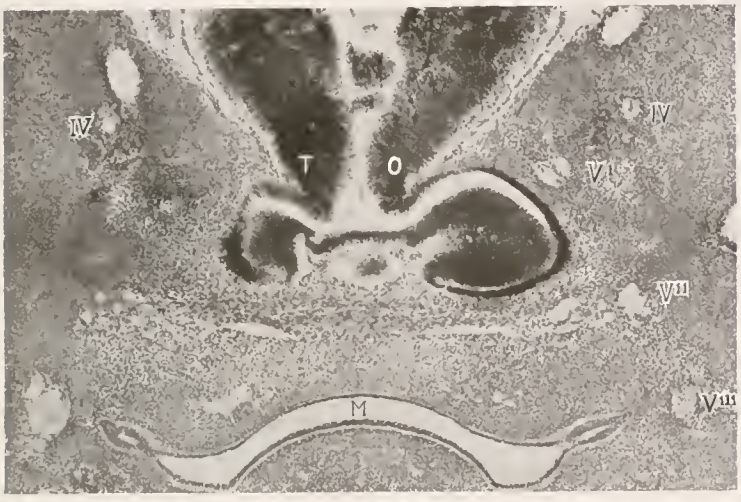



FIG. 1.

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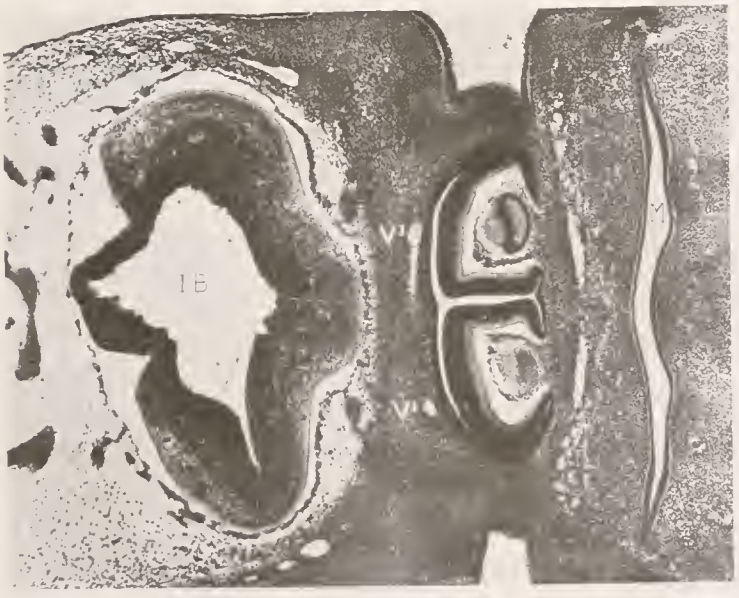


FIG. 4.

FIG. 3.

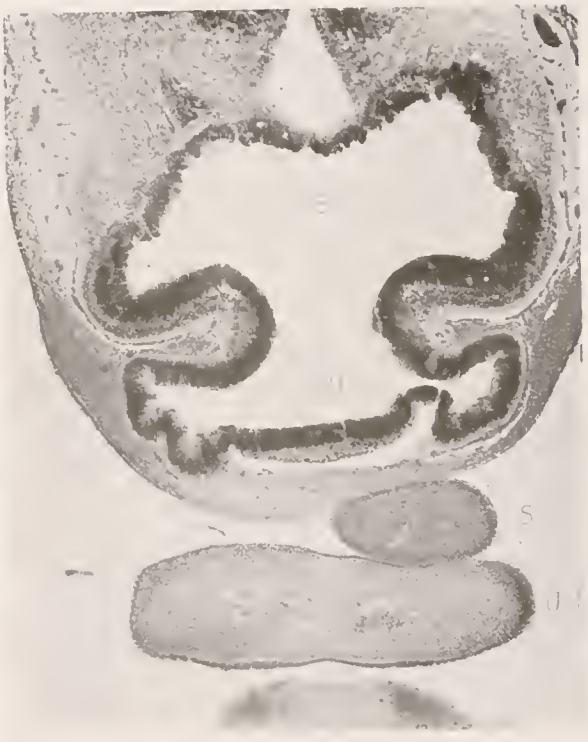


FIG. 5.

CONTRIBUTIONS TO EMBRYOLOGY, No. 16.

# QUANTITATIVE STUDIES ON MITOCHONDRIA IN NERVE-CELLS.

By Madge DeG. Thurlow.

One plate.

# QUANTITATIVE STUDIES ON MITOCHONDRIA IN NERVE-CELLS.

By Madge DeG. Thurlow.

The hope of being able to establish a sound foundation for investigation into cellular physiology has inspired most of the work on cell constants. Perhaps the best known of these is the nucleus-cytoplasmic ratio of Hertwig (1902), which has already proved of great value in the investigation of changes in nerve-cell activities. On account of the reawakening of interest in mitochondria, the attention of investigators has been drawn in recent years to the cytoplasm. This is not surprising, as structural changes resulting from experimental variations are evident in the cytoplasm, for it is here that most of the products of differentiation are laid down and readjustments in response to changes in the environment take place.

Though the study of mitochondria has been carried far along many different lines, up to the present time no attempt has been made to place these cytoplasmic structures upon a quantitative basis. With this object in view the present work was undertaken, making use of a favorable method of technique. No attempt was made to establish a ratio between mitochondria and cytoplasm on the basis of relative volumes, the number of mitochondria per unit volume of cytoplasm being the basis of comparison. This relationship has served well as an adequate foundation for comparison of various nerve-cells and is thought to be of particular value in the case of the nerve-cell, inasmuch as it possesses no other cytoplasmic constituent lending itself to quantitative study; for the Nissl substance differs so widely in form and density that it is absolutely impossible with our present methods of technique to estimate its amount with any degree of accuracy, and the significance and form relations of the neurofibrils are not clear.

The original plan of the investigation was to determine whether by comparisons of the mitochondrial content of known motor and sensory cells there was a distinctive difference between cells of these categories. With this purpose in view, quantitative estimations of mitochondria in the nuclei of origin of the cranial nerves were made. The results were disappointing, in that they showed that the mitochondrial content could not be used as a basis of classification for motor and sensory cells, but they did show something that was not known before, viz, that the number of mitochondria per unit volume was constant for the nucleus of any cranial nerve.

## MATERIAL AND METHOD.

The animal selected was the white mouse, and the observations were confined to the nuclei of the cranial nerves. The nuclei of the IX and XI nerves were not included in the investigation, owing to the difficulty of ascertaining with absolute certainty what cells constituted these nuclei in the nucleus ambiguus.

The method of fixation and staining has been described by Cowdry (1916*a*). The mitochondria, which take the fuchsin, appear as discrete, bright-red granules sharply outlined against a background of light-green Nissl substance, and, with proper optical and lighting facilities, may be readily counted.

Quantitative estimations were made by carefully counting the mitochondria occurring within a field of constant area. Such a field was obtained by placing within the ocular a glass disk upon which a single square had been ruled according to the method of Isaacs (1915). The actual area of the optical field covered by the square was determined by the stage micrometer and found to be 19.78 square micra. The thickness of the sections was 4 micra; hence the volume of the field counted could easily be determined, and from that the amount of mitochondria per cubic millimeter was calculated.

The lenses used were the Zeiss apochromatic 1.5 mm. objective and Zeiss No. 6 compensating eyepiece. Constant conditions of illumination were obtained by the use of the 40-watt Mazda lamp. A mechanical stage was employed. All counts and drawings were made under uniform conditions of magnification and illumination.

In order to avoid inaccuracies due to possible minor differences in thickness of the sections, the same number of fields was counted in every nucleus occurring in any one section. For example, in the same section may be found cells of the cochlear and vestibular nuclei of the VIII, of the VI, VII, and the mesencephalic nucleus of the V. If four fields were counted in one section in mesencephalic cells, four fields of all the other nuclei were counted in that section.

## OBSERVATIONS.

Studies were carried out upon five brains which had been cut into partial serial sections. Only such nuclei as were well stained in each series were investigated and the results tabulated (table 1).

TABLE 1.

| Nucleus. | Series 1259 | Series 1348 | Series 1237 | Series 1260 | Series 1257 | Remarks. |
|---|---|---|---|---|---|---|
| Mesencephalic of V. | 22.6 | 22.5 | 21.7 | 22.9 | 22.1 | |
| VI | 23.5 | ..... | 20.7 | 17.4 | ..... | |
| VII | 15.1 | 15.7 | ..... | 15.0 | ..... | Series 1259, male, weight 9 grams, age 33 days. |
| VIII (vest.) | 17.6 | ..... | ..... | ..... | ..... | Series 1348, female, weight 13.5 grams, age 61 days. |
| VIII (vent. coch.) | 18.8 | 18.9 | ..... | ..... | ..... | Series 1237, male, weight 10 grams, age 25 days. |
| X (motor) | ..... | 14.1 | ..... | 14.7 | ..... | Series 1260, male, weight 8.5 grams, age 31 days. |
| XII | ..... | 15.2 | 14.3 | 15.4 | 14.3 | Series 1257, female, weight 9 grams, age 35 days. |
| III | ..... | ..... | ..... | ..... | 17.3 | |
| IV | ..... | ..... | ..... | ..... | 22.9 | |

The above figures represent the average number of mitochondria present in the field counted, the volume of which was 79.12 cubic micra. It will be noted that there are many gaps in the series. This is due to the fact that, owing to the great difficulty of cutting perfect serial sections 4 micra in thickness, some of the nuclei are missing. Again, perfect definition of outline of the mitochondria is required before they can be counted, and since it is impossible, even with the most expert technique in staining, to attain this ideal in every section, some of them could not be used.

At first 150 fields were counted in each nucleus and the average was taken, but as there was never any wide variation in the figures obtained for different fields the number was limited to 50, and later to 10. Most of the averages recorded here were based on 10 field counts—never on less. Lists were kept of the number of mitochondria in each field as they were counted, and such variations as the following were noted: In 35 fields observed in the VI nucleus the numbers ranged from 20 to 26; in 10 from the mesencephalic the range was 20 to 24; in 10 from the VII it was 14 to 17. This uniformity ran through all the nuclei of all the series, with a few exceptions that will be mentioned later.

In order to determine the percentage error in the total, 10 fields were recounted in several cases and the error estimated, and it never amounted to more than 1.3 per cent. This maximum occurred in the cells most closely crowded with mitochondria; in cells having few mitochondria the recounts showed no error. Table 2 contains the detailed results of specimen counts and recounts.

TABLE 2.

| Nucleus VII (motor). | | Nucleus VI. | | Nucleus XII. | Nucleus V (mesencephalic). | Remarks. |
|---|---|---|---|---|---|---|
| Original count. | Recount. | Original count. | Recount. | Original count. | Original count. | |
| 15 | 16 | 25 | 24 | 17 | 22 | Original counts of four nuclei are given. The figures illustrate the uniformity of the mitochondrial content of the individual nuclei and are typical of all the nuclei counted. In the case of nuclei VII and VI figures for the recounts are also given (right-hand columns). These show little variation from the original count. In making the recounts, the slide was not moved, in order to have the limits of the field unchanged, the recount being made immediately after the original count. |
| 15 | 16 | 25 | 26 | 15 | 24 | |
| 16 | 15 | 21 | 22 | 15 | 22 | |
| 16 | 17 | 27 | 26 | 15 | 25 | |
| 15 | 16 | 24 | 26 | 15 | 23 | |
| 14 | 15 | 28 | 27 | 14 | 23 | |
| 14 | 14 | 23 | 24 | 14 | 21 | |
| 16 | 17 | 22 | 20 | 14 | 24 | |
| 16 | 14 | 21 | 23 | 16 | 22 | |
| 17 | 16 | 25 | 23 | 17 | 22 | |

Care was taken to avoid cells whose limits could not be clearly defined. Inasmuch as the cell processes and the neuroglia possess mitochondria, it was necessary to choose sharply outlined cells, in order to eliminate errors due to counting mitochondria outside the cell-body.

It has been stated that the number of mitochondria in the cells of the same nucleus is quite constant, but that there are some exceptions. In one animal, in the VI nucleus some cells had fewer mitochondria than others, but as these appeared normal in other respects they were counted, the variation ranging in this instance from 13 to 21. The greater number, however, contained about 17 per field. In the case of the cochlear nucleus there was a group of cells dorsal and somewhat lateral to what appeared to be the main body of the nucleus. The dorsal group corresponds to current descriptions of the dorsal cochlear nucleus, and the larger group to the ventral cochlear nucleus. The cells of the dorsal nucleus contained practically no mitochondria, were slightly smaller, and possessed a cytoplasm clearer than those of the ventral nucleus. Only the cells of the ventral nucleus were included in the count, for it was felt that cells which

showed such constant and specific morphological differences could not be included in the same functional category.

Upon referring to plate 1 it will be seen that there are striking similarities between nuclei of different functional categories. For example, the mesencephalic nucleus (fig. 7) of the V (regarded as sensory by most authors—as Johnston, 1909, and Willems, reviewed by Donaldson, 1912) and the motor nucleus of the IV (fig. 4) have the same number of mitochondria per unit volume of cytoplasm; the visceral motor nucleus of the VII (fig. 1) and the somatic motor nucleus of the XII (fig. 5) have the same average; so, too, have the vestibular of the VIII (somatic sensory, fig. 2) and the motor of the III (fig. 6). Not only do nuclei of different categories agree in the number of mitochondria they contain, but those of the same category disagree, the numbers for the somatic motor nuclei being 14, 17, 20, and 22 mitochondria per unit volume of cytoplasm. Comparisons of the nuclei of the same general classification are made in table 3.

TABLE 3.

| Somatic motor nuclei. | | Visceral motor nuclei. | | Somatic sensory nuclei. | |
|---|---|---|---|---|---|
| Cranial nerves. | Average number of mitochondria per field for all cells counted. | Cranial nerves. | Average number of mitochondria per field for all cells counted. | Cranial nerves. | Average number of mitochondria per field for all cells counted. |
| III | 17.3 | X | 14.4 | V | 22.3 |
| IV | 22.9 | VII | 15.3 | VIII (vest.) | 17.6 |
| VI | 20.5 | | | VIII (vent. coch) | 18.8 |
| XII | 14.8 | | | | |

No visceral sensory nuclei were studied. It may be seen that no distinction can be made between motor and sensory nuclei on the basis of their mitochondrial content.

In order to have some idea of the enormous number of mitochondria in the cells of the nuclei of the cranial nerves and of the really tremendous variations in number of mitochondria in cells of the different nuclei whose number of mitochondria per field vary only from 14.1 to 22.5, the results of some determinations as to the number of mitochondria per cubic millimeter of cytoplasm will be given. In nerve-cytoplasm containing 22.5 mitochondria per field, such as that of the cells of the mesencephalic nucleus (fig. 7), the number of mitochondria per cubic millimeter would be 284,378,159; the number for the cytoplasm of the cells of the nucleus of the X nerve (fig. 8), containing 14.1 per field, would be 178,210,313.

This study has been very carefully controlled. In the first place, several observers have briefly called attention to the fact that mitochondria differ in form not only in different nerve-cells, but also to some extent in the same cell. Nicholson (1916), working in this laboratory, has made a careful study of these morphological variations in mitochondria in the nerve-cells of the white mouse, the same form which I have studied. He, however, worked with cells other than those of

the cranial nerves, where the mitochondria are, for the most part, granular, as seen by the illustrations. Working particularly with the anterior-horn cells and the large cells of the reticular formation, he describes mitochondria which are filamentous, the filaments varying in length, some being so short as to be almost identical with the granular forms, as one proceeds from the periphery to the nucleus.

Naturally the question would arise as to whether, under these circumstances, quantitative variations such as those recorded in table 1 have any real significance. It is obvious that the method which I have adopted of counting the mitochondria would have to be accompanied by very accurate measurements in order to yield reliable information of the relative as well as actual amount of mitochondria in cells in which their size and shape differ to any appreciable extent. Filamentous mitochondria, though occurring in the cell processes, are rarely found in the cell-bodies of the cranial nerves and, since my observations are confined to the cell-bodies, these filamentous mitochondria do not constitute a source of error. A few of them are homogeneous throughout, but most can be resolved by careful focussing into rows of discrete granules, fairly uniform in size. For the work in hand this was a great advantage, for by counting the granules a more accurate index of the *amount* of mitochondria was obtained than would have been possible by counting the filament as a unit. Though the method of counting here used is not ideal in the case of cells characterized by great dimorphism on the part of their mitochondria, it does yield reliable results when care is taken to restrict its use to cells in which the size and shape of the contained mitochondria are practically uniform, as in the cells of the nuclei of the cranial nerves.

Despite the fact that the granules were of the same size, if there were lack of uniformity in their distribution, an accurate estimation of the mitochondria for any one cell would be impossible unless all the mitochondria in that cell were counted. It is because of their practically uniform distribution that the amount in any one field can be used as typical for the whole cell. Counts were made of different fields of a cell in one section, also of fields selected from the same cell in successive serial sections; and the numbers were practically identical. It is true that there is usually a slight crowding of mitochondria in the axon hillock, with a tendency for them to be arranged in filaments or rows of granules along the long axis of the cell-process (figs. 3, 4, and 5). In the canalicular system, which in these preparations shows white, there are no mitochondria; where they do seem to occur in the canals the appearance is due to their presence in the thin layer of cytoplasm surrounding the canal. Especially in figures 1 and 5 there appear to be large areas free from mitochondria. This is explained by the fact that all the drawings were made in one optical plane; on a different focus mitochondria would have appeared in the cytoplasm, which is now free from them. No variations were noted in the density of distribution of mitochondria other than those just mentioned. Any minor unevenness in the distribution of mitochondria would be obviated by the fact that the square used in counting is relatively large, taking in an expanse of cytoplasm extending in most instances from the nucleus to the periphery of the cell.

Again, it has been shown that mitochondria vary in their solubilities in acetic acid (Regaud, 1910; Nicholson, 1916). In view of this fact objection might be made that the variation in mitochondrial content in different types of nerve-cells was an artefact produced by the solvent action of the reagents used. Such criticism of the results obtained in this investigation is invalid, for no acetic acid was used in the preparation of the specimens, nor was there any other solvent for mitochondria involved in the fixation or staining. The formalin which was used as a fixative for the mitochondria was neutral, and the long mordanting in the bichromate prevented their subsequent solution in alcohol, although their solubility in alcohol does not seem to be marked. Hence there is no error in technique which could account for the striking variation in amount.

## DISCUSSION.

Having established the certainty that there is, in the nerve-cells of the medulla of the species used, a definite amount of mitochondria per unit volume of cytoplasm, there remains to be determined the functional significance of such numerical variation. Other investigators have, in their work on the central nervous system, determined various cell ratios, among which might be mentioned the nucleus-plasma ratio. Dolley (1914) has found that the resting nerve-cells of corresponding type for the same species have a constant nucleus-plasma ratio which is altered temporarily through functional depression or permanently through functional senility. Having once established this constant, he could study pathological changes following experimental conditions. Donaldson (1911) found variations in the water content of the nerve-cells accompanying functional changes. He makes no further statement than this: the variation in water percentage of the nerve-cell is an index of functional activity.

The relationship between number of mitochondria and volume of cytoplasm is another such constant. In the animals studied the number of mitochondria per unit volume of cytoplasm was found to be constant for corresponding cells, not only of the same animal, but also of different individuals of the same species (table 1). In nerve-cells of the same type, therefore, we have a cell constant which is definite for animals of the species studied and which can be used for observations of pathological conditions resulting from experiment. That mitochondria do react to conditions which affect the cell has been demonstrated by several authors, as Policard (1910 and 1912), Homans (1915), Scott (1916), and others.

These observations may be given another application, viz, to the doctrine of neurone specificity. It would be reasonable to suppose that even if all the mitochondria were identical, such definite and constant variations as are here recorded would be closely associated with a definite and constant functional differentiation. Combined, however, with this quantitative difference are qualitative differences (Nicholson, 1916), and this combination serves to strengthen the theory that the activity of the nerve-cells themselves differs in some way. It would be rather extreme to assume that cells differing specifically with respect to so con-

stant an element in their cytoplasm were functionally identical, for although as yet the rôle played by mitochondria in nerve-cells is unknown there is evidence that they play an important part in other cells. Cowdry (1916*b*) has discussed briefly the literature bearing on the relations of mitochondria to cell metabolism. From such evidence it is safe to assume that they are not an unimportant constituent of nerve-cells and that their constancy in amount in the normal cell is definitely associated with its normal activity.

## CONCLUSION.

There is a constant number of mitochondria per unit volume of cytoplasm in normal nerve-cells of a corresponding type in the mouse. This constant differs for nerve-cells of different types. Sensory and motor cells can not be distinguished on the basis of their mitochondrial content. The significance of constant variations can not be interpreted with our present meager knowledge of the rôle played by mitochondria, but there is support for the theory that nerve-cells are functionally differentiated in the evidence here advanced of their constant difference with respect to the number of mitochondria they contain.

Finally, I desire to express my cordial thanks to Dr. E. V. Cowdry, who kindly suggested the problem and provided the material for the investigation.

Baltimore, *May 31, 1916.*

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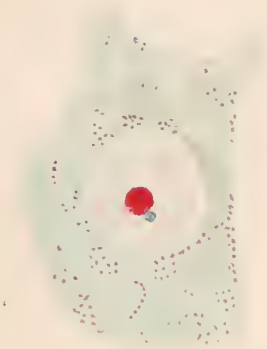
## EXPLANATION OF PLATE.

The following drawings were made with the aid of a camera lucida, a 1.5 mm. apochromatic Zeiss objective, and No. 6 compensating Zeiss ocular. All drawings are at a magnification of 1,650 diameters.

The Nissl substance appears as a background of bluish-green masses, between which are the unstained canals of the canalicular system. Against this background the mitochondria appear as bright-red dots. All cells were drawn without changing the focus, so that sometimes the mitochondria are clumped within the Nissl substance and sometimes within the canals, although this latter appearance is due to their presence in the thin layer of cytoplasm surrounding the canals. If the focus were changed sufficiently mitochondria would occupy the spaces now free. The counting, however, was done by focussing through the whole depth of the section. Some of the mitochondria seem to be smaller and less definite than others; this, in all the drawings, is due to the fact that they were slightly out of focus, yet sufficiently clear to be included in the drawing. Where they occur as chains of granules they are probably broken filaments. The granules, when brought clearly into focus, are of approximately the same size.

### Plate 1.

1. A typical cell of the motor nucleus of the VII cranial nerve. Note its similarity to the cell of the XII nucleus (fig. 5), both with respect to the appearance of the Nissl substance and the number of mitochondria, the number per unit volume of cytoplasm for both being 15.
2. A cell from the ventral cochlear nucleus of the VIII nerve. The Nissl substance here is a diffuse violet, and the mitochondria stain more intensely than those in the other nuclei.
3. A motor cell from the nucleus of the VI nerve.
4. A motor cell from the nucleus of the IV nerve. It will be noted that there appear to be more mitochondria in the cytoplasm of the cell of the mesencephalic nucleus (fig. 7) than in this, although the counts show that the number per field is practically the same. This is due to the fact that the cytoplasm of the mesencephalic cell is much more transparent, lacking the great clumps of relatively opaque Nissl substance which characterizes the IV nucleus cell; and so in a single optical plane, mitochondria may be seen to a greater depth in the mesencephalic cell. Since focussing was done throughout the depth of the section, the Nissl substance did not interfere with the accuracy of the counts.
5. A motor cell from the nucleus of the XII nerve.
6. A motor cell from the nucleus of the III nerve.
7. A sensory cell from the mesencephalic nucleus of the V nerve. The mitochondria appear closely crowded, even on one plane.
8. A cell from the dorsal motor nucleus of the X nerve.



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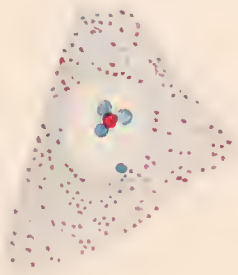

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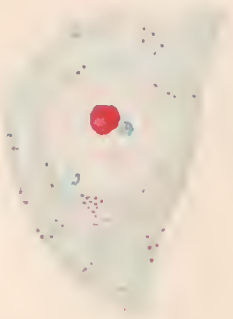

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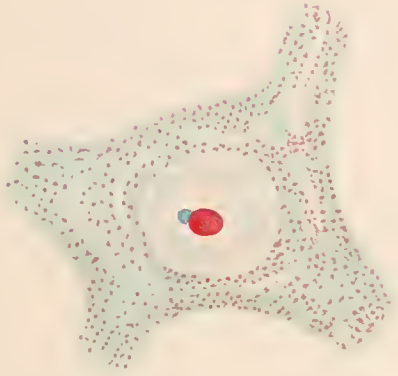

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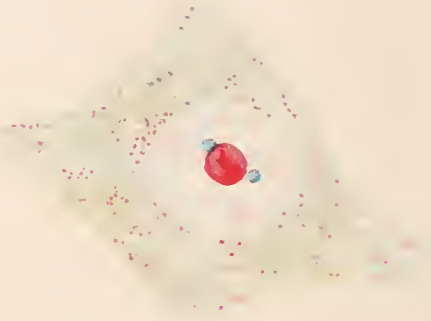

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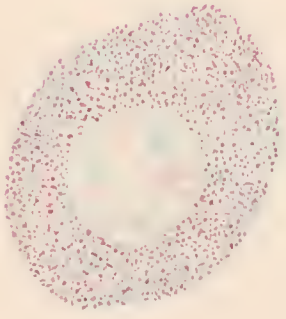

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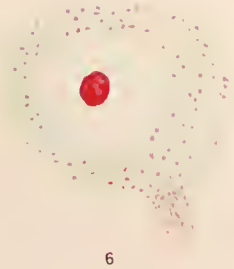

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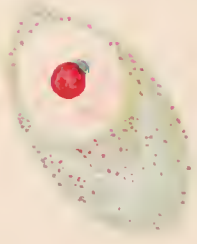

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CONTRIBUTIONS TO EMBRYOLOGY, No. 17.

# DEVELOPMENT OF CONNECTIVE-TISSUE FIBERS IN TISSUE CULTURES OF CHICK EMBRYOS.

By Margaret Reed Lewis.

Two plates.

# DEVELOPMENT OF CONNECTIVE-TISSUE FIBERS IN TISSUE CULTURES OF CHICK EMBRYOS.

By Margaret Reed Lewis.

Up to the present time the study of fixed and stained preparations of embryos has failed to decide the question of the origin of the connective-tissue fibers. This is partly because the methods used necessarily coagulate and distort the delicate cell processes and also the intercellular substances, and such results readily lend themselves to various interpretations by different investigators.

In the following observations on tissue culture an attempt has been made to study the formation of the connective-tissue fibers directly within the living tissue, and not only within living tissue, but within tissue which has developed in an environment entirely free from fibrin or any substances other than those within the cell which coagulates upon fixation.

The pieces of tissue to be explanted for the tissue cultures were washed through several changes of Locke's solution until such fibrogen as was present had become coagulated within the pieces themselves before they were explanted. The medium thus remained free from fibrin, for no fibrin network was observed in the medium of any preparation. In order to see whether the substance forming fibrin would be dissolved out from the explanted piece and deposited as a fibrin network in the medium of the culture, a few cultures were made in which the explanted pieces of tissue were not carefully washed. No fibrin network was found in these cultures, even after many days. However, in such preparations a delicate network formed over the growth (not within the growth) upon fixation, showing that some substance had been dissolved out from the explanted piece, which coagulated upon fixation. Although this network did not resemble fibrin network, or the delicate processes from the cells, or even the fibrous tissue itself, all such preparations were discarded in order to avoid any possible chance of error.

By this method it is taken for granted that such connective-tissue fibrils as form in the tissue-culture growths arise from the cells, either as a secretion formed by the cells and deposited in the form of fibrils and fibers, or from the transformation of the cytoplasm of the cell itself. As will be seen later, while there is evidence of a possible secretory activity of these mesenchyme cells, as Renaut (1904) and Renaut and Dubreuil (1906) have claimed, due to the presence of the "grains de segregation," or the so-called vacuoles of Lewis and Lewis (1915), nevertheless, in these tissue cultures, the connective-tissue fibrils formed by a transformation of the cytoplasm of the cells.

Tissue culture is not an entirely satisfactory method for the study of any highly differentiated tissue, owing to the fact that the cells which migrate out from the explanted piece and later increase by division attach themselves so closely to the cover-slip and become spread out in such a thin layer that the differ-

entiated structure loses its characteristic appearance. Also, the cells of the new growth have a tendency to migrate away as individual cells instead of developing into a differentiated tissue composed of numerous cells. In all probability the cells do not de-differentiate and become more embryonic, as has been claimed by Champy (1913) and others, but simply lose their characteristic differentiated appearance, due to their changed shape and position. This is interestingly shown by a study of smooth muscle-cells (plate 2, fig. 6). Where the cells are attached closely to the cover-slip they no longer contract and the myofibrils appear as irregular bundles composed of numbers of delicate fibrils (plate 2, fig. 6). However, where the cell is not so closely attached to the cover-slip it continues to contract, and in this case the myofibrils are arranged into the characteristic fibrils. Taking the possible loss of the characteristic appearance of the differentiated structure into account, the very thin and largely spread-out living cells of the tissue culture furnish an excellent means for the study from day to day of certain structures of the cell.

Just how much differentiation can take place in such cells in these tissue cultures is difficult to state. Certainly in a few cases, where the mesenchyme growth at 48 hours was composed of quite undifferentiated cells, this growth, when kept alive by frequent baths of fresh solution, did develop definite connective-tissue fibrils. Muscle fibers have been observed to become more differentiated; but in the case both of the muscle fibers and connective tissue there is some continuation of function, as the muscle fibers frequently contract, and the connective-tissue growth also occasionally contracts back around the explanted piece and later grows out again.

Fibrils did not develop in many of the cultures of connective tissue, owing to the fact that the cells remained spread out as individual cells until the death of the culture. In the few cultures that were kept alive for a sufficient length of time, and in which the connective-tissue fibers did develop, they could be clearly seen and studied in the living preparation from day to day, and their development could be traced from the earliest delicate fibril within the exoplasm of the cell to the more adult fibers, which appear to be free from the cells.

## PREVIOUS WRITINGS ON THE LIVING CONNECTIVE TISSUE.

Whether the connective-tissue fibers arise within the cells or from an intercellular substance is still an open question. The weight of evidence seems to be in favor of a cellular origin, although certain text-books of histology present the question almost wholly from the intercellular point of view.

There are many reviews of the literature on both sides of the question (Fleming, 1891; Spuler, 1896; Mall, 1901; Rothig, 1907), and also various text-books, and since the technique used by other investigators is so different from that employed in the following observations no effort will be made to take up in detail the various papers upon the origin of the connective-tissue fibers.

While many observers have studied teased preparations of connective tissue, Boll (1872) was the first to study the development of the connective-tissue fibrils entirely from the living cell. Boll made his cultures by teasing out a few of the

cells of the tissue to be studied and placing them in a hanging drop of amniotic fluid. Although he did not obtain any growth of the teased-out cells, he was able to observe the connective-tissue fibrils in connection with the cells throughout the different stages of the development of the connective-tissue fibers, and he became convinced, by this study of the living cells, that the fibrils had their origin within the cells and continued through the exoplasm of one or more cells. Boll studied carefully the following tissues:

Arachnoid of chick embryo of 4 to 19 days' incubation.
Subcutaneous tissue of chick embryo of 7 to 17 days' incubation.
Cornea of chick embryo of 4 to 21 days' incubation.
Tendon of chick embryo of 7 to 21 days' incubation.

He concluded that in all of these tissues the connective-tissue fibers originated from the cells.

In the study of the connective tissue by the tissue-culture method, preparations such as those studied by Boll were used, and also many others, in which the tissue was either teased out, or flattened out, or suspended in a hanging drop of Locke's solution instead of amniotic fluid. Figures similar to those given by Boll were frequently observed (plate 1, figs. 2, 4, and 5), and his observations were corroborated. However, in all such preparations it is impossible to eliminate the possibility of fibrin or some other intercellular substance taking part in the formation of the fibrils, and for this reason these observations will not be given below, although they undoubtedly show the connection of the fibrils with the cells.

As no cultures containing fibrin have been studied, nothing can be said at this time in regard to Baitsell's observations (1915), by means of which he shows that certain fibers which resemble the connective-tissue fibers may form in a fibrin clot in the presence of a piece of tissue of a chick embryo after various periods of time. In the development of the embryo there can be no question of fibrin playing any part in the formation of the fibrous tissue, since, so far as is known, fibrin is not present in the uninjured tissue. Whether the cells of the embryo possess the power of secreting a substance which may act in the same manner as the injured cell to produce the formation of fibrin, or whether the connective-tissue cells in the developing embryo act directly upon the plasma, are questions which Baitsell does not discuss. He quotes the following experiments of Loeb, from Adami:

"When a drop of uncoagulated lymph is placed between two glass slides, the mere act of pulling one slide over the other leads to the appearance of fibrils, which grow in length and bulk; which like those of connective tissue are not only intracellular, but actually traverse cell bodies situated in their path; which show themselves first in immediate connection with the cells, the cells as we now hold liberating an enzyme that determines the modification of the more soluble protein into a precipitated or coagulated modification. But the lines of the precipitation are evidently along the lines of strain."

These experiments of Loeb are in a way comparable to those of Baitsell, except that in Baitsell's experiments the strain is brought about by the shrinkage of the plasma clot. It seems rather difficult to draw any conclusions in regard

to the manner of the formation of the connective-tissue fibrils in the embryo from results which are so obviously due to injured cells as are those of Loeb's experiments. However, there is a striking resemblance between the fibrous tissue obtained by Baitsell by means of a modification of the fibrin clot and the fibrous tissue of the embryo.

Baitsell's (1916) paper on wound-healing, in which he finds that very shortly after a wound has been made in the skin of a frog, fibrin fibers, which resemble connective-tissue fibrils, are deposited, and that these fibers persist and take part in the formation of the cutaneous tissue, opens the exceedingly interesting question as to whether what takes place in the process of wound-healing can be in any way comparable to the behavior of normal developing tissue.

So far as can be gathered from Isaacs's (1916) incomplete report of his observations upon the living connective-tissue fibers, his results correspond more or less with those of Loeb—that is, that various strains cause the intercellular substance to form fibrillæ. Just what part the cells play in this formation it is difficult to understand. Isaacs does not say that the cells form an enzyme, as Loeb claims, but states that the movements of the connective-tissue cells probably effect the distribution of the material through chemical or other action and cause the fibrillated structures of the adult fiber. From Isaacs's brief report it is evident that he had performed numerous experiments with the living connective tissue, and it is hoped that his complete paper will clear up many points.

Ferguson's (1912) observations upon the living connective-tissue cells in the fins of fish embryos are extremely interesting, since by his method the connective-tissue cells were studied under entirely normal conditions. Since there has been some question as to whether fibers actually exist or whether they are merely coagulations of a colloid within the tissue due to abnormal conditions, it is interesting to note that Ferguson describes fibers as well as cells as existing in the living embryo. He found, by the aid of preparation stained by Bielschowsky's method, that the fibers arise within the cell. Unfortunately he was not able to see the fibers in the embryonic cells of the living embryo and to determine whether they become separated from the cells, or how this takes place. His observations upon the movements of the connective-tissue cells show that the round and stellate cells may move up to and stretch along a fiber as a very thin, long spindle cell, and in a few cases he observed such a spindle cell to become stellate again.

Ebeling (1913) has for a period of two years or more kept alive certain of the cultures started by Carrel. The growth of these cultures consists mainly of connective tissue, and Ebeling claims that connective tissue may have a permanent life outside the organism when properly cared for. The method of keeping the culture alive is as follows: The entire culture in its plasma clot is freed from the cover-slip and washed in Locke's solution to remove any waste products and is then cut into four or more pieces, and each of these pieces is again explanted into a drop of fresh plasma. This procedure is carried out every other day; although many of the cultures die, a few survive and grow, and are again explanted as described above. In all probability there is no differentiation of the connective tissue into fibrils or fibers, as Ebeling describes the growth as though it consisted

of undifferentiated mesenchyme, which is what would be expected in any tissue that proliferates as rapidly as this tissue necessarily must. It would be interesting to see whether, if one of the cultures were kept alive without further explanation, it would again differentiate after a certain equilibrium of proliferation had been reached.

Some workers have claimed that certain substances present in the medium of tissue cultures prevent the growth of the connective tissues. For example, Walton (1914) states that liver extract inhibits the growth of adult mammalian connective tissue in plasma cultures, and Russel (1914) claims that gentian violet, in solution of 1/20,000 in the medium of tissue culture, prevents the growth of connective tissue but not of endothelium. The reason for this neither writer explains, nor does either state what structure of the cell is affected by the substance so that the cells do not grow out, or whether the medium may simply not attract the cells to migrate and that the cells themselves are uninjured.

Thus a review of the literature on the living connective-tissue cells shows that the study of the living tissue has not presented decided proof as to whether the connective-tissue fibers arise from the cells or are formed from an intercellular substance. Evidence is presented on both sides, and the question remains as completely at a dead-lock as when the observations were confined to fixed and stained preparations.

## OBSERVATIONS IN GENERAL.

A few general observations as to what takes place in the tissue cultures of connective tissue are given here in order to show what factors influence the growth of the fibers.

Cultures from the subcutaneous tissue of chick embryos of various ages were made in the usual manner (W. H. and M. R. Lewis, 1915; M. R. Lewis, 1916). Lewis and Lewis have shown that while the cells in the new growth in tissue cultures are under somewhat abnormal conditions as regards environment and nourishment, nevertheless they are actively growing cells which undergo normal division and which grow out as definite types of cells—that is, nerve-cells, muscle-cells, heart-muscle cells, endoderm of the intestine, epithelial cells of the skin, and connective-tissue cells.

As has been stated, no fibrin is present in the medium, and no substance which coagulates.

The subcutaneous tissue can be removed as a thin, transparent sheath from the skin of chick embryos of 10 days or older. It proved difficult to isolate the subcutaneous tissue from embryos younger than 8 days; and in these embryos a piece of skin or one of the deeper skin fascias or the arachnoid tissue was used for explanation. The connective tissue of embryos less than 8 days old is composed of cells without definite fibrils; that of embryos of 11 days and over contains definite bundles of fibers. The new growth from an explanted piece of subcutaneous tissue of embryos of 8, 9, and 10 days proves very satisfactory for the study of the development of the fibrils. The growth can be kept alive and healthy by frequent baths of fresh Locke's solution, plus 10 per cent bouillon, plus 0.25 per cent dextrose; and fibrils begin to develop in the new growth in from 48 to 72

hours and continue to develop until the growth is about 6 days old or over. Cultures of the subcutaneous tissue from an 11 or 12 day chick embryo also prove very satisfactory for study, for not only is the new growth available for study, but the explanted piece itself is so thin that the cells and fibers can be observed even with the oil-immersion lens.

The fibers in the explanted piece were not observed to grow either in length or bulk, and after a period of two weeks they remained much the same as when explanted. In no case was a fiber observed to pass out from an explanted piece over the new growth; such a fiber always remained curled up within the explanted piece.

New fibrils begin to develop in the new growth from an explanted piece of tissue from an 8 to 12 day chick embryo shortly after the new growth is 24 hours old, and definite bundles of fibrils may be developed when the growth is 5 or 6 days old. These fibrils develop more quickly in growths from the older embryos of 10 to 12 days than in those from the younger embryos of 8 to 10 days.

The new growth from the subcutaneous tissue is extremely sensitive and reacts to all sorts of changes in its environment, by contraction. Frequently while a membrane of connective tissue was under observation it would begin to contract from the outer edge of the growth and draw in towards the explanted piece. This contraction might stop at any period or it might continue until the entire new growth had contracted close to the explanted piece. The explanted piece was never observed to contract. The relaxation after such a contraction was exceedingly slow, and frequently a contraction that had taken no longer than 2 to 5 minutes required for relaxation from 1 to 6 hours. In fact, the process did not resemble relaxation, but rather a growing-out again of the new growth. Often, coincident with the contraction, there occurred a rolling-back of the edge of the growth, and in this case when the cells migrated out again many of them became changed in their relative positions. Thus it is evident that a decided strain is present during the development of the fibrils, though there is no fibrin and (so far as can be seen) no substance which coagulates surrounding the cells. Whether this strain or tension (often exhibited by the contraction above described) may in any way influence the separation of the fibrils from the cytoplasm of the cell, it is impossible to state. It was not certain that a preparation which contained well-developed fibrils had not contracted during the development of the fibrils. However, it can be definitely stated that no substance formed into fibrils during contraction, as might have been expected from the experiments of Loeb and of Isaacs. The new growth, when relaxed after such a contraction, never contained any suddenly formed fibers or fibrils, and such fibrils as were present were in very much the same state of development as that in which they were before the contraction took place. Also, many membranes in which no fibrils ever developed possessed the power to contract, and did contract more than once.

From these general observations it is evident that the fibers which form in the tissue cultures must arise from the cells; and since the cells are spread out in a thin layer the process of development of the fibers can be observed in the living cell undisturbed by any manipulation.

## OBSERVATIONS IN DETAIL.

The growth from a piece of chick embryo of 6 to 8 days' incubation is usually in the form of a membrane closely attached to the cover-slip, and is composed of large, flat cells, either connected by numerous cytoplasmic processes (plate 1, fig. 1) or else crowded together so that the delicate processes from cell to cell are lost and a more definite cell-wall appears (plate 2, fig. 10). The growth from older chick embryos may also sometimes have the appearance of a membrane, especially where the cells are spread out in a thin layer along the cover-slip. When such a growth is treated with silver-nitrate stain the membrane becomes marked with more or less definite cell-walls, according to the amount of crowding of the cells (plate 2, fig. 10). Such a membrane has been described by Clark (1914), where the connective tissue is stimulated to grow out over a very smooth surface, which Clark interprets as showing that under certain conditions the connective-tissue cells may become transformed into endothelium. While the pattern which appears with the silver-nitrate stain is in many ways characteristic of endothelium, still growths from older chick embryos (8 to 10 days) in these tissue cultures exhibited the characteristic activities of connective-tissue cells, and in some cases fibrils were formed within the cytoplasm of the cells (plate 2, fig. 10).

The growth from an 8 to 10 day chick embryo usually has the appearance of a reticulum of cells (plate 1, figs. 7 and 8). Some of these cells are of the large, flat, stellate type, having processes on all sides, in which may develop bundles of fibrils, which pass in more than one direction through the cells (plate 1, figs. 7, 8, and plate 2, fig. 10); others are cone-shaped—*i. e.*, while the cell-body may have several short processes, most of the cytoplasm is drawn out into one long process (plate 1, fig. 2, and plate 2, fig. 4). Both the granular and the clear cytoplasm is continued out into the one long process, which practically always extends in the direction from which the cell has migrated, and although in many cases it continues back as a delicate thread, passing as many as twelve or more cells, it has always a protoplasmic end, either free or closely attached to another cell. These long processes usually contain mitochondria and other granules scattered along their length, and never in any case have they been observed to change into connective-tissue fibers. In many "film preparations" of the subcutaneous tissue studied while alive, such long, delicate processes have been observed to extend along the side or through the middle of a bundle of fibrils. This is probably due to the fact that, through some stress, the cell has been drawn out into this shape, either from migration or manipulation, and the fibrils are those which were originally in the exoplasm of the cell.

During the beginning migration (1 hour after explantation) of the cells in the explants from older chick embryos (10 to 15 days), when certain of the cells first begin to migrate it is seen that they are drawn out into exceedingly long and delicate processes which ramify in all directions, as though their cytoplasm had extended a great length along the fibers of the subcutaneous tissue (plate 1, fig. 3). As the cell continues to migrate towards the periphery of the explanted piece or out into the culture medium, these long processes are drawn into the cell, until finally

it becomes stellate in form and later divides by mitosis and may again develop long and delicate processes among the cells of the new growth.

From a study of this cell (plate 1, fig. 3) and of the cells shown in plate 1, figure 8, and plate 2, figure 4, it can be seen that in the embryo, where the growth is in all directions rather than in a flat plane (as in tissue cultures), a section must necessarily cut many of these delicate processes and cause the appearance of a network of isolated protoplasmic threads between the cells, because the connection of these threads with the cells to which they belong is not shown in the section.

Typical spindle-shaped cells never appeared in pure cultures of connective tissue, but always in those which contained muscle-cells; and in every instance a typical spindle-cell could be identified as a muscle-cell.

In certain of the explanted pieces spindle-cells were observed, but these appeared to be due to the pull which had been put upon the tissue during manipulation, for frequently parallel bands of fibers extended along these cells.

In some preparations from a 10-day chick embryo the cells were connected by so many delicate processes that a network of these processes was formed between them, which would have been difficult to identify as cellular in origin had it not been for the fact that during the mitosis of one of these cells all the delicate network connected with the cell was partly drawn into it, and the space around the cell became free from network (plate 2, fig. 5). It thus became clear that the protoplasmic network between these cells was not extracellular in origin.

The fibrils appeared first (after 24 hours' growth) as slightly more refractive lines within the cytoplasm of the individual cells (plate 2, figs. 1 and 9). The mitochondria were frequently stretched along these delicate lines; by careful study, however, it was seen that the mitochondria did not take part in the formation of the cellular fibrils, but that even though they stretched for a certain distance along a fibril they later separated from it.

As the growth became older (48 to 72 hours) the cells become more and more densely connected by delicate processes with cells at a distance; and the refractive line of the primitive fibril appeared more and more within the cell and became partly gathered into bundles at one point or another (plate 2, figs. 1 and 2). The cellular cytoplasm became separated into an endoplasm—that is, the granular cytoplasm which contains mitochondria, fat, neutral red granules, etc.—which immediately surrounds the nucleus, and an exoplasm, or the clear, non-granular cytoplasm of the more remote surfaces of the cell (figs. 10 and 13).

The delicate fibrils of the cytoplasm continued from one cell to another, usually through the exoplasm of the cell processes (plate 1, fig. 9 and plate 2, fig. 2) and appeared in the living cell as clear, slightly more refractive lines of exoplasm, extending from one cell to another, and frequently across or through the exoplasm of one or more cells.

As the fibrils developed from day to day the bundle became more definite and more independent of the cytoplasm of the cells, until finally it extended as a slender, clear fiber across several cells (plate 2, fig. 3), and except in cases where the

individual fibrils can be traced into a cell, the bundle, or fiber itself, appeared quite independent of the cytoplasm of the cells (plate 2, figs. 2 and 3).

No mitosis was observed in cells whose exoplasm was actively developing into fibrils during the time in which the exoplasm contained the fibrils. Mitosis, however, continued, and many cells were seen to undergo mitosis in regions where other cells were forming fibers. So far as can be determined from these observations, the cell may again undergo mitosis after the bundle of fibers has become independent of the cell cytoplasm. Whether in such cases the cell actually separates itself from that part of its exoplasm which has been differentiated into fibrils or whether it simply divides the undifferentiated cytoplasm and meanwhile remains attached to the differentiated exoplasm (or fibers) could not be determined. However, the cells which contributed fibrils to a fiber bundle gradually increased in number and extended over a wider territory, and the bundle became differentiated into a more and more definite fiber (plate 2, figs. 1 and 3).

The study of the living cell, as well as of the fixed preparation, led to the idea that the fibers became more and more separated from the cells, although it is quite possible that they may merely continue through the exoplasm and become more definite, on account of the separation of the cells. Certainly in no case, in these tissue cultures, did the fiber become so well developed that the ending of the various fibrils which made up the fiber could not be traced into the exoplasm of a cell (plate 2, figs. 2 and 3). No completely differentiated fiber was observed throughout its development, although in a few instances, where the cultures were kept in a healthy condition for several weeks, fibers which resembled those of an 18-day chick embryo were developed. It seems probable that the development of these fibers was by a continuation of the process described above.

Certain preparations which had been carefully studied during their growth and development were fixed and stained, and from these preparations most of the photographs and drawings have been made. A few of the living cells were drawn on successive days, and although it was frequently impossible to determine the exact cell drawn the day before, at least a cell in its near neighborhood was taken.

## MITOCHONDRIA AND THEIR RELATION TO THE CONNECTIVE-TISSUE FIBRILS.

One of the most convincing arguments in favor of the view that the fibrils arise within the cytoplasm of the cells is the fact that frequently a few mitochondria are seen along a primitive bundle of fibrils (plate 2, fig. 2) and that occasionally a few are found isolated within a well-developed bundle of fibrils in the primitive fiber (plate 2, fig. 3). So far as is known, mitochondria can not exist extracellularly.

The mitochondria of the cells of the growth from a 6-day to 10-day chick embryo are usually of several types; that is, the granular, the short-rod, and the long-thread or filament type. The greatest number are filaments. Mitochondria are scattered throughout the cytoplasm and occasionally along the network of cell processes between the cells, and they may be arranged in a row along a cytoplasmic process (plate 1, fig. 6). In a few instances a single filamentous mitochondrium has been observed to lie along the length of such a process (plate 2, fig. 3). A mitochondrium may be stretched along a fibril in such a way that in a fixed preparation it would be difficult to determine whether or not it took part in the formation of the fiber. However, a study of the living cell shows that the mitochondria retain all their characteristic activities. They continue to bend, twist, and migrate, with the result that a mitochondrium, even though stretched for a time along a fibril so that it appears to be part of the fibril, very soon bends and later may move away. Mitochondria arranged in a row along a cell process do not necessarily remain there, but may migrate into the body of the cell again.

In the older cultures the cell processes are usually free from mitochondria. In these cultures the mitochondria are more or less centralized around the nucleus—*i. e.*, within the endoplasm of the cell.

There is present in certain of the cells another structure, which stains in the manner characteristic of mitochondria with the various mitochondrial stains—red with Bensley's anilin-fuchsin methylene green stain (plate 2, fig. 7), black with iron hematoxylin (plate 1, fig. 6), and purple with Benda's method. This structure is in the form of a deposit along certain lines of the surface of the cell (plate 1, fig. 6), and is not present in the cell in its early development, but appears later along the edge or on the surface of the cell, and in certain cells, although not usually in those of subcutaneous connective tissue, frequently seems to be associated with the formation of fibrils. It seems probable that it is this structure rather than mitochondria which Meves (1910) had under observation when he described the formation of the fibrils of the tendon as taking place from the mitochondria after they had become arranged along the surface of the cell. In the stained preparation this structure definitely resembles the mitochondria, and it would be difficult to determine whether mitochondria take part in its formation. However, the living cell shows clearly that the structure is along the surface of the cell and that the mitochondria do not take part in its formation. Also, while the structure is fixed and stained by the same methods which fix and stain the mitochondria, it is not necessarily destroyed by the agents which destroy mitochondria, but may be present in

preparations in which the mitochondria have been destroyed. It is very similar to the structure which forms the fibril of the muscle-cell (plate 2, fig. 6).

Mislavsky (1913) was able to differentiate a plasma fibril as well as mitochondria in the kidney tubule cells. He found that while the plasma fibrils stretched entirely across the cells as straight lines, the mitochondria did not pass to the walls of the cells.

In the cultures studied mitochondria did not fuse into strands or become arranged in rows to form the connective-tissue fibrils. In all of these observations, while the mitochondria at times remained caught within a bundle of fibrils, the fibrils themselves originated from the exoplasm of the cell.

## OTHER GRANULES AND "GRAINS DE SEGREGATION" OF THE CONNECTIVE-TISSUE CELL.

The connective-tissue cell ordinarily contains very few fat globules, and frequently none at all. When present they are small, round, highly refractive globules, which usually lie near the nucleus and which stain in the manner characteristic for fat.

In addition to the mitochondria granules in the cells, there are a number of small, round granules, which can be distinguished from the granular type of mitochondria only by the rapidity of their movements and by certain vital dyes. These granules stain blue with pyrol-blue, purple with brilliant cresyl-blue, and red with neutral red. In the fixed preparation they frequently take certain of the mitochondrial stains, and especially do they take the same purple color as the mitochondria with Benda's stain.

The vacuoles of Lewis and Lewis (1915) correspond more or less with the *grains de segregation* of Renaut (1904, 1907) and Renaut and Dubrieul (1906), which these observers found to stain with neutral red and which they claimed formed fibrils through a secretory activity of the cell. These bodies are present in the connective-tissue cell, sometimes in large numbers (plate 2, figs. 2 and 3), but so far as could be determined they take no part in the formation of the fibrils.

## TENDON.

Only a few growths of cells which could be definitely identified as tendon-cells took place, and in these growths the formation of the fibrils occurred in a manner somewhat different from that of the formation of the fibrils of the subcutaneous tissue. The tendon-cells were arranged as narrow, elongated cells, more or less parallel, and the fibrils developed as clear lines along the surface of the cells. These delicate lines joined into bundles from one cell to another in markedly parallel lines (plate 2, fig. 8). In preparations stained with Mallory's connective-tissue stain the fibrils stained blue.

## AMNION.

A study of the fibrils of the amnion was undertaken in order to see whether the observations of Péterfi (1914) could be corroborated in tissue cultures. Péterfi observed vacuoles within the epithelial cells of the amnion, and concluded from his preparations that these vacuoles fused together and became more numerous in the cells of the amnion of chick embryos of from 3 to 5 days' incubation. According to Péterfi, the walls of these vacuoles contain a substance which is different from the remainder of the cytoplasm, and as the walls fuse together they form a network of elastic fibers over the epithelial cells of the amnion of a chick embryo of 7 days' incubation. My observations on the amnion in tissue cultures did not show this. The epithelial cells contained varying numbers of vacuoles, most of which stained with neutral red, though a few remained unstained. Fibrils are formed, but, so far as can be determined from these observations, they are formed from the exoplasm of the cell, regardless of the vacuole, in practically the same manner as are the fibrils of the connective-tissue cells (plate 2, fig. 9).

## CONCLUSIONS.

1. The connective-tissue fibrils begin to develop in the subcutaneous tissue of chick embryos of from 9 to 10 days' incubation, and appear as well-developed fibers in the subcutaneous tissue of a 12-day chick embryo. The new growth from explanted pieces of subcutaneous tissue from chick embryos of 8 to 10 days' incubation proved the most satisfactory for the study of the connective-tissue fibers.

2. The cut fibers which are present in the explanted piece of 11-day to 15-day chick embryo subcutaneous tissue do not grow either in length or bulk in the tissue cultures.

3. The new growth of connective tissue is exceedingly sensitive and reacts by a contraction of the cell from the outer edge in towards the explanted piece. This contraction does not cause the formation of fibers in the new growth.

4. Fibers are not present in the 24-hour growth from even 12-day to 15-day chick-embryo tissue, but develop in the cells of the new growth from delicate fibrils in the exoplasm of the cells after 24 hours.

5. The fibrils developed as delicate lines of the exoplasm of the cell; they became gathered into bundles which passed from cell to cell, and the bundles later passed over or through the exoplasm of several cells as a definite fiber. The fibers never became so adult that the individual fibrils which make up the fiber could not be traced into the cytoplasm of some cell, whether near or distant from the main body of the fiber.

6. Although the new growth, when closely attached to the smooth coverslip, often takes the form of a membrane, and although this membrane exhibits the cell pattern which is characteristic for endothelium when treated with silver nitrate, nevertheless there is no evidence that these cells have become endothelial cells; they still retain the characteristics of connective-tissue cells, and many form fibrils.

7. The mitochondria do not take part in the formation either of the fibrils or of the fibers in these cultures of connective tissue.

8. There was no evidence that the fibrils are formed by a secretory activity of the *grains de segregation* (vacuoles) of the connective tissue.

9. The fibrils of the epithelial cells of the amnion appeared to form in the same manner as those of the subcutaneous tissue—*i. e.*, from the exoplasm of the cell, and not from the fusion of the walls of the vacuoles.

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## EXPLANATION OF PLATES.

### Plate 1.

1. Photograph of the membrane of connective tissue of 48-hour growth from a tissue culture of a piece of stomach of a 6-day chick embryo. 4 oc. and 4 mm. lens. Zeiss.
2. Photograph of cells and fibers in a film preparation of subcutaneous tissue of an 18-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.
3. Camera-lucida drawing of a living cell from a culture of subcutaneous tissue of 14-day chick embryo, 3-hour growth. 6 oc. 4 mm. lens.
4, 5. Camera-lucida drawings of living cells in a hanging-drop preparation, after Bell, of subcutaneous tissue from a 14-day chick embryo in Locke's solution. 4 oc. 4 mm. lens.
6. Retouched photograph of 48-hour growth from arachnoid tissue of 7-day chick embryo. Os. vapor, iron hem., 4 oc. oil-imm. lens.
7. Photograph of 48-hour growth from leg of 8-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. 4 mm. lens.
8. Photograph of 48-hour growth from subcutaneous tissue of 10-day chick embryo. Os. vap., iron hem. 6 oc. 4 mm. lens.
9. Photograph of 48-hour growth from heart of 7-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.

### Plate 2.

1. Camera-lucida drawing of cell, showing primitive fibrils in the cytoplasm. 48-hour growth from subcutaneous tissue of 9-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
2. Camera-lucida drawing of cells and fibrils united into bundles. 72-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
3. Camera-lucida drawing showing cells with fibrils within the cytoplasm and also fibers. 120-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap , iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
4. Camera-lucida drawing of cells from 24-hour growth of leg of 8-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens.
5. Camera-lucida drawing of cell undergoing mitosis in 48-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens.
6. Camera-lucida drawing of smooth muscle-cells, showing myofibrils from 48-hour growth of amnion of 5-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.
7. Camera-lucida drawing of cell from 24-hour growth of 6-day chick embryo, showing deposit along surface of the cell, which is stained like mitochondria with Bensley's aniline fuchsin, methylene green stain. 4 oc. oil-imm. lens.
8. Camera-lucida drawing of tendon-cells from 72-hour growth of muscle of 9-day chick embryo. Zenker's fixation—Mallory's stain. 4 oc. oil-imm. lens.
9. Camera-lucida drawing of epithelial cells of 48-hour growth from amnion of 5-day chick embryo. Iodine-vapor fixation—Mallory's stain. 4 oc. oil-imm. lens.
10. Camera-lucida drawing of thin membrane of connective tissue from 72-hour growth of 10-day chick embryo. Silver nitrate and Ehrlich hematoxylin. 4 oc. and oil-imm. lens.

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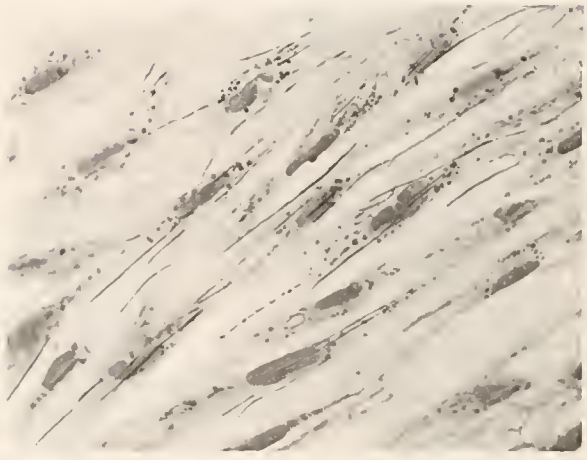

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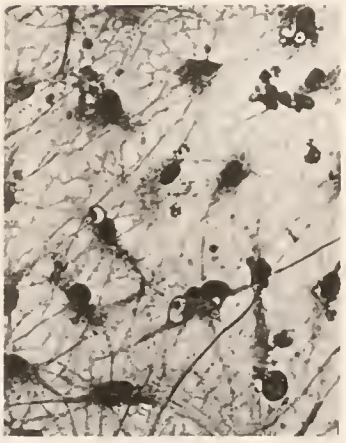

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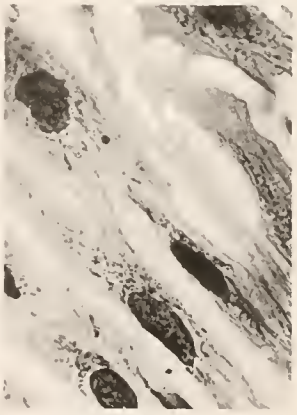

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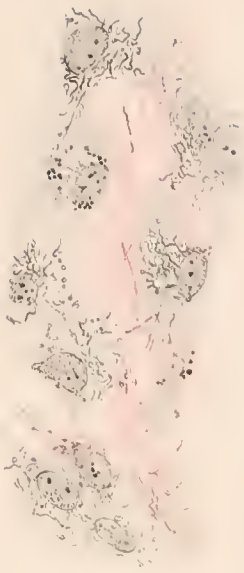

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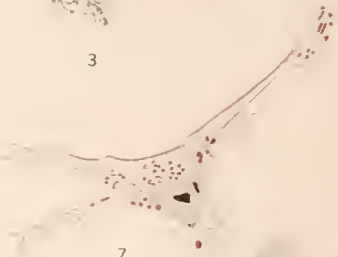

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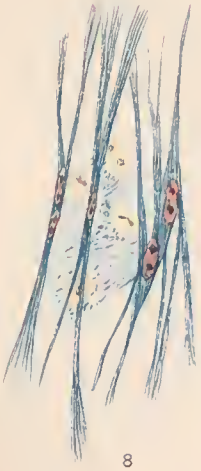

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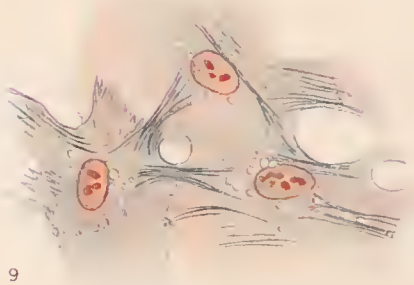

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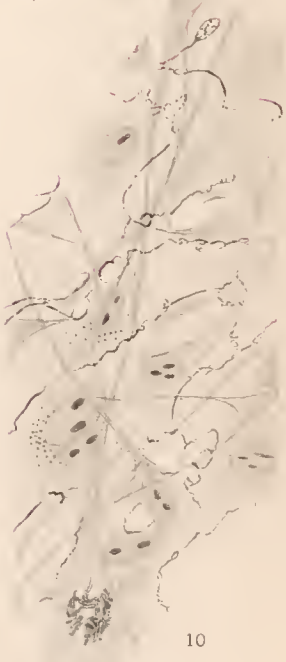

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CONTRIBUTIONS TO EMBRYOLOGY No. 18.

# ORIGIN AND DEVELOPMENT OF THE PRIMITIVE VESSELS OF THE CHICK AND OF THE PIG.

By Florence R. Sabin.

With seven plates and eight figures in the text.

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# ORIGIN AND DEVELOPMENT OF THE PRIMITIVE VESSELS OF THE CHICK AND OF THE PIG.

By Florence R. Sabin.

## INTRODUCTION.

In this paper is given an account of the primitive vessels of the chick and of the pig, as made out by injecting living embryos, and, in the case of the chick, as seen growing in the embryo. Such studies must necessarily be accompanied by the study of sections. In the case of the mammalian embryo I have made injections in earlier stages than had been done heretofore and in the case of the chick I have carried the method of injection to the earliest stage in which it is possible. Below the stage at which they can be injected, the vessels of the chick can be studied in the living blastoderm by a technique which has developed out of the method of tissue-culture introduced by Harrison. The chick thus offers unusually valuable material for the study of vascular problems, as it is possible to use both the method of injection and that of direct observation of the living embryo in the same stage.

In the course of this study two fundamental ideas have been under consideration. The first concerns the most essential question in connection with the vascular system, namely, the relation of differentiation and growth of endothelium. According to one theory there is a limited period for the differentiation of angioblasts out of undifferentiated mesenchyme, and after this period all new blood-vessels arise from the growth or proliferation of older angioblasts. This theory seems to me to have the weight of evidence. The second theory is that angioblasts continue to differentiate out of mesenchyme indefinitely. If the former theory is correct and the period of differentiation of endothelium is a limited one, the fundamental problem concerning the early blood-vessels is to determine which differentiate and which are formed from preceding vessels. In practically every embryo chick observed, up to a certain stage new angioblasts can be seen differentiating and joining the older angioblasts, but the phenomenon becomes less and less frequent as older stages are studied. In the living embryo the aorta itself can be seen to differentiate out of mesenchyme, and at the stage when the heart begins to beat every chick shows a few isolated angioblasts along the mesial border of the aorta, which will be seen to join the aorta if the specimen be watched for a short time. I have some evidence also that some of the primitive vessels along the neural tube differentiate out of mesenchyme, the process being observed in the living embryo. On the other hand, one can watch the growth of the entire wall of a vessel by cell-division in the living embryo and the formation of new vessels from the walls of old vessels; so that the study of the early blood-vessels is gradually becoming a more exact problem, namely, the determination for each vessel, whether it differentiates *in situ* or develops from preceding vessels. My present material is not adequate for the solution of this question, but throws some light upon it.

The second question—which has proved of great interest—is the definition of the terms *artery*, *vein*, and *capillary* as they are used for the embryo. In the study of the vessels of the embryo particular stress should be laid on the time when circulation begins. That there is a very extensive development of blood-vessels before there is any circulation of the blood due to the beat of the heart was well known to the earlier embryologists; for example, to von Baer and later to His. Moreover, the heart beats for a considerable time before it starts any circulation. It is known that the blood-vessels spread over the body in definite and constant sheets of capillaries, and in these primitive vessels, after the circulation has begun, a vessel may serve as an artery for a time and then be reduced to a capillary plexus, in which the direction of the circulation is entirely different from that of the circulation of the original artery. Such a vessel, for example, is the subintestinal artery of the pig, which arises in a capillary plexus around the caudal end of the primitive gut and carries blood out to the arteries of the yolk-sac, where it must again pass through a capillary bed before it returns to the heart. This artery becomes broken into a capillary plexus in the wall of the gut, which makes new connections with branches of the omphalo-mesenteric veins within the wall of the mesentery, so that its blood, instead of flowing away from the embryo to the membranes, flows within the embryo toward the heart.

Again, a vessel may serve for a time as a vein in the return of blood to the heart and may subsequently receive new arterial connections and become an arterial plexus, with the direction of the flow of blood entirely changed. Such a vessel is the so-called vena capitis medialis. This is a primitive vessel along the hindbrain, which in the chick in the second and third days of incubation serves as a vein for the forebrain and midbrain, but as an arterial capillary trunk for the hindbrain; that is, it carries mixed blood and is the only vessel of the hindbrain, representing its entire capillary bed. Early in the fourth day it receives new arterial connections, a new vein develops to carry the venous blood for the forebrain and midbrain, and the primitive vascular channel of the hindbrain breaks into a capillary plexus in which the direction of the current of blood is at right angles to the direction of the original current. From these two examples it must be clear that in the study of the primitive vascular system it is very important to understand the function of the vessels at each stage of development, and any presentation of the vascular system which overlooks this point and is dominated wholly by the pattern of the vessels of the adult becomes difficult to follow and may be misleading. In the question of nomenclature a decision has to be made between two theories—that is, whether the vessels are to be named according to the function they perform at any given stage or whether they are to be named according to the vessels for which they form the primordia. If the latter method is chosen it must be remembered that a given vessel of an embryo often disappears entirely in giving rise to new vessels—for example, the primitive vessel of the hindbrain.

In this study I shall use terms as consistently as possible, in the following manner: By the term *artery*, in reference to an organ, I mean a vessel which brings blood to that organ but does not form any part of its capillary bed, and I have colored such vessels red. By the term *vein* I mean a vessel which carries blood from

an organ to the heart, provided it does not serve as the capillary bed for that organ or break up into another capillary plexus. Such vessels I have colored blue. All other vessels I have indicated in gray, and shall try to trace the complicated changes which such vessels undergo, serving at times as arteries, veins, or capillaries, or as vessels with a double function. This method of nomenclature is therefore based on the function of the vessel at the stage when it exists. It takes into account the very shifting course of the blood, as the vessels develop in the embryo better than does the method of making a too early identification of the adult vessels in those of the embryo. This usage of terms serves to restrict especially the term *vein* as applied to the embryo. The meaning of the terms *artery* and *vein*, as applied to the embryo, will become more clear in the discussion of individual vessels.

## METHODS.

As has been stated, in this study I have followed two methods: first, that of the injection of embryos, and second, the method of studying the living blastoderm in the case of the chick in a hanging-drop preparation. A general account of the methods of injecting embryos will be found in my paper on the azygos veins published as "Contribution to Embryology, No. 7," by the Carnegie Institution of Washington in 1913. All of the injections of young embryos are made by blowing ink into the vessels through a very fine canula.

To inject the young chick the shell is opened and the embryo exposed to a strong light under a binocular microscope. A few drops of warm Locke's solution are placed on the blastoderm, and the vitelline membrane is removed. By the time the chick has 14 somites the sinus terminalis, or marginalis, is well developed in the edge of the area vasculosa and can easily be punctured with a fine canula. Nevertheless it is not easy to obtain complete injections of the blood-vessels of the chick through the veins until the embryo has about 16 somites. In stages between 9 and 16 somites more complete injections can be made by puncturing the aorta directly. This is a very interesting point in connection with the time of the beginning of the circulation. I shall show that, though the heart commences to beat about the time the tenth somite is forming, the circulation does not begin until about the stage of 16 somites. From the time the circulation begins it is easy to get complete injections by blowing a little ink into the vitelline veins and allowing the heart to pump the ink through the vessels of the embryo. If total specimens are desired it is well to dilute the ink one-half, so that the superficial vessels will not become so dense as to obscure the deep ones. I shall discuss in this paper the effects of injection in the embryo before the circulation has begun.

The earliest chick embryo which I have injected was one of 9 somites; and I believe this stage to be about the youngest to which the method is applicable. At the stage of 6 somites the dorsal aorta is in the stage of a plexus of angioblasts, many of which are still solid cells. This plexus of cells gradually acquires a lumen and becomes the aorta during the stages of from 6 to 9 somites. All stages up to 18 or 19 somites can be studied to great advantage in hanging-drop preparations.

Direct injections into the aorta can be made in the following manner: When the embryo is placed in a strong light the myotomes are of course very plainly visible, and along their lateral border can be seen a faint opaque streak, which is the intermediate cell-mass or nephrotome. Between the nephrotome and the lateral border of the myotomes is a thinner line which is more transparent. The canula is then introduced between the lateral border of the myotomes and the intermediate cell-mass, with the point toward the head of the embryo. If the canula enters the aorta, and only a slight pressure is used, there need be no extravasation at the point of puncture. After the embryo has been injected it is fixed by dropping Bouin's mixture on the specimen while it is still on the yolk. It is kept flooded with the fixing agent, and is not removed from the yolk until it is well hardened.

In regard to the injection of young mammalian embryos there are a few special points in technique which are of interest. In order to identify the young embryo pig the mucosa is examined carefully for long strings of chorion, which are so inconspicuous that they are more readily found by running the finger over the mucosa than by sight. These strings of chorion are then very carefully coiled on a glass slide or piece of filter paper until the embryo is found. In the case of the pig, I have never succeeded in puncturing the veins on the yolk-sac or the umbilical vein. The latter is so large that it might be punctured if it contained enough corpuscles to render it visible. The aorta, on the other hand, is readily punctured opposite the mid-body region. Here in early stages the two aortæ seem slightly dilated, or later are fused into a single vessel. The needle is introduced ventral to the myotomes. The injection mass in every case was india ink. Silver nitrate seems to damage the tissue much more markedly in very young embryos than in later stages. After injection the pigs were fixed in Carnoy's mixture of absolute alcohol 6 parts, chloroform 3 parts, and glacial acetic acid 1 part. They were then placed in 80 per cent alcohol, dehydrated in graded alcohols, and cleared by the Spalteholz method of benzine followed by oil of wintergreen.

The study of the blastoderm of the living chick embryo in a hanging-drop preparation depends on the methods originated by Harrison and developed by a large group of workers—Burrows, Carrel, M. R. Lewis and W. H. Lewis, and others. In 1912 McWhorter and Whipple applied the method to the study of the growing blastoderm of the chick, which was its first application to the entire embryo, so far as I am aware. These investigators mounted the blastoderm in clotted plasma and used the method to test the question as to whether blood-vessels arise from fusion of isolated vesicles. In 1913 Brachet published a study on the growth of a mammalian embryo in a hanging-drop preparation.

In studying the blastoderm of the chick by the method of the hanging-drop I have followed the technique of Margaret Reed Lewis, of growing the embryo in Locke's solution. In this way the embryo can be kept growing for several hours, and the cells which are nearest the cover-slip can be seen with great clearness and followed with an oil-immersion lens. The embryo is removed from the yolk and placed in a dish of warm Locke's solution and the vitelline membrane

and most of the yolk removed. It is more difficult to study the blastoderm with the dorsal surface against the cover-slip than from the ventral aspect, because the ectoderm does not adhere to the glass as well as does the endoderm, and it is necessary to have the embryo very flat in order to see the cells with higher powers. In regard to the blood-vessels, it is of course preferable to study the embryo from the ventral aspect, since the blood-vessels are nearer the endoderm than the ectoderm. In the study of the developing blood-islands it would be very advantageous to have the dorsal surface of the embryo against the cover-slip, as the blood-islands are farther dorsal and because the cells of the ectoderm over the area opaqua are much thinner than the cells of the endoderm over the same area. The cells of the endoderm of the area opaqua are so thick and so filled with globules of yolk that one can seldom focus through them in the living embryo. From the ventral aspect there is also an area of the axis of the embryo that it is very difficult to study with high-power lenses, namely, the portion of the axis just caudal to the head-fold, because the heart lifts the embryo from the cover-slip and the cells can then be studied only with dry lenses.

All of the chicks have been fixed in Bouin's mixture of saturated aqueous picric acid 75 parts, formalin (40 per cent) 20 parts, and glacial acetic acid 5 parts, for about 12 hours. They are then placed directly in 60 per cent alcohol. In the case of the chicks which have been growing on the cover-slip it is very necessary to have the embryo stick to the cover-slip throughout the fixation and dehydration. If the specimens are to be mounted *in toto*, they are mounted on the same cover-slip on which they were growing. If they are to be embedded and cut they can be removed from the cover-slip after they have been cleared in the oil of wintergreen. The specimens do not become as brittle in the oil as in xylol. In the blastoderms which are kept on the cover-slips it is possible to watch the effects of dehydration much more accurately than with free specimens. The edge of the specimen around the outer margin of the area opaqua clings very closely to the cover-slip; in fact, in mounting the embryo strands of tissue are pulled out which dry slightly and help the specimen to remain fixed. If the specimens are put into alcohol weaker than 60 per cent this outer margin will stick to the cover-slip, but the entire area pellucida will become free from the cover-slip and swell into a bleb. The space beneath fills in with fluid, and in the subsequent dehydration there is an uneven shrinkage which distorts the tissues. Thus, weak alcohol or water, or a dilute stain, macerates and swells the tissues and the subsequent shrinkage distorts the cells. On the other hand, if the specimens are placed directly in alcohol as strong as 60 per cent, a plexus of cells, which has been studied in the living specimen, can be readily identified in the fixed specimens. If the specimens are to be stained *in toto* they must be placed in a stain sufficiently strong so that the tissues will not macerate before they react to the stain. From such an experience one should avoid washing embryos in water and should also avoid the use of the lower grades of alcohol. I have used several changes of 60 and 65 per cent alcohol to eliminate the excess of picric acid. The dehydration can be done by changes of 5 per cent. If it is carried out too rapidly the specimens will crack, but the shrinkage with the higher grades

of alcohol is by no means as marked as in the case of older embryos. The early embryos are injured much more by maceration due to weak alcohol than by shrinkage due to too rapid dehydration in the stronger grades. The specimens are all cleared by the Spalteholz method of benzine followed by oil of wintergreen. Specimens can be embedded from oil of wintergreen if they are passed directly from the oil to a mixture of the oil and paraffin. The tissue does not become too brittle to cut even after remaining in the oil for a year or two.

## VASCULAR SYSTEM OF THE CHICK.

### GENERAL ACCOUNT OF THE VASCULAR SYSTEM OF THE CHICK UP TO STAGE OF 14 SOMITES.

In the study of the origin of the vessels of the chick I shall begin the detailed account with the stage of 6 somites. The study of the blastoderm in a hanging-drop preparation offers a valuable method for a study of the early stages. In the stages of the early somites there is a plexus in the area opaqua which, by the older embryologists, Pander, von Baer, Remak, and later by His, was identified as the forerunner of the blood-vessels. Basing his studies on those of von Baer and Remak, His gave a description of the origin of blood-vessels which remains the foundation of our knowledge upon this subject (1868, pages 95 to 100). He described the first appearance of blood-vessels, or, as he later termed them, angioblasts, as occurring just before the appearance of the somites. He stated that the vessels began as a plexus of angular or spindle-shaped solid cells in the area opaqua. These cells from the beginning were in the form of a plexus (Gleich von Anfang an ein geschlossenes Mosaik, page 98). The plexus was at first made up of solid cells without a lumen, and grew by processes of solid spindle-shaped cells, exactly similar to those which formed the original network. This plexus was in a definite layer—the vascular layer (das Gefässblatt) of Pander.

The vascular layer, His said, consisted not only of solid angular cells, but also of elements having a yellow color, or, in other words, it gave rise not only to blood-vessels but also to blood-cells. He regarded it as of the greatest importance that the first appearance of vessels was in the area vasculosa before the heart formed, and that these vessels arose entirely independently of any circulation. He then noted that the plexus of solid cells became transformed into vessels, the exact method of the transformation being impossible to determine; but that as the solid cells became the walls of vessels, their cytoplasm became less granular and their nuclei flatter. He then described the approach of the blood-vessels toward the axis of the embryo by means of the same type of solid processes which formed the original plexus and found that they approached the axis in two zones: first, opposite the myotomes, and secondly, along the splanchnopleure in the region opposite the future emphalo-mesenteric veins—that is, over the ventral surface of the two amnio-cardiac vesicles.

His noted that over the region of these amnio-cardiac vesicles there was a double sheet of vessels which approached the axis of the embryo, a more scanty sheet

in the somatopleure, and a more abundant sheet in the splanchnopleure. Because of the development of the head-fold and the heart, it was impossible that the approach of the vascular plexus should be uniform over the entire length of the embryo. For example, the most cephalic part of the head became cut off from direct lateral connection with the embryonic membranes, and the vessels which approach the heart gradually rotated from the direct transverse direction to an oblique angle. He then noted that the dorsal aorta developed in the mesial edge of the plexus of angioblasts of the area vasculosa along the line of the lateral edge of the myotomes. From these observations he concluded that the vessels of the embryo are derived from the vessels of the membranes, and that the portion of the axis which can not be seen to receive the plexus of primitive angioblasts from the membranes receives its vessels by a growth of the plexus which has already invaded the embryo at other places.

In following the differentiation of the vascular area by improved methods whereby one can watch the living cells growing under an oil-immersion lens, it is astonishing how accurate is this description of His, which must have been made by far cruder methods. To his description must be added that with finer methods it is seen not only that the plexus out of which the aorta develops is the border of the common plexus of the entire area vasculosa, but that new cells differentiate along the axis of the embryo as well, so that angioblasts differentiate over the entire zone from the outer edge of the area opaqua to the margin of the future aorta along the lateral border of myotomes. Thus His's description must be extended to include a differentiation of new angioblasts in the axis of the embryo itself.

In the living blastoderm over the area opaqua, the endoderm-cells are so thick and so filled with yolk that the development of the blood-vessels and the blood-cells beneath them can be followed only with great difficulty. In the area pellucida, on the other hand, the endoderm is thin, and during the periods when the endoderm cells are not dividing they are so clear that it is easy to focus through them.

At the stage of 6 somites the head-fold is well formed and the amnio-cardiac vesicles have met in the mid-ventral line. Along the axis of the embryo there is a zone of dense tissue radiating from the primitive streak and from the embryo cephalic to the streak. This denser mass of tissue divides the area pellucida into an inner thicker zone containing the axis of the embryo and an outer thinner zone. In sections it is clearly seen that this denser zone is due to the further development of the cœlom nearer the embryo. Over the cephalic part of the denser zone the cœlom has a wide lumen, and both its ventral mesoderm and the endoderm are thicker than the same membranes farther lateralward. This is very plainly shown in Duval's Atlas, plate XIV, figure 218, in the zone extending outward from his letter *b*. Farther caudalward in this dense band, opposite the undifferentiated myotomes and the primitive streak, there is no cavity of the cœlom, and its dorsal and ventral mesoderm are fused and form a dense mass of cells. This entire thicker zone is difficult to study in the living chick, but the whole outer margin of the area pellucida is clear and the cells are so thin that

one can readily focus through them from the endoderm to the ectoderm, and see every cell of the entire zone. This is true, however, only when the endoderm is not dividing. The endoderm cells divide as a whole, and during the entire phase of cell division they are so opaque that it is impossible to focus through them. The phase requires about an hour, and to study the vessels beneath it is necessary to await until the cells become clear again.

In the entire outer margin of the area pellucida, at the stage of 6 somites, there are two plexuses. The dorsal plexus, which is the developing cœlom of this area, appears to be composed of very large and flat vessels. Distinctly ventral to this plexus of the cœlom is another plexus, much less abundant and made up of solid bands of cells, which are angioblasts. An exceedingly important point, which can be determined with great distinctness in the living specimen, is that the plexus of angioblasts connects by many tiny filaments with the plexus of the mesoderm of the cœlom, but never connects by filaments with the endoderm. In sections the angioblasts of the vascular layer often touch the endoderm, but in the living embryo they are always separate. The living specimens also bring out very sharply the fact that the entire layer of angioblasts is distinctly ventral to the plexus of the mesoderm; in other words, the term *vascular layer* of Pander is an appropriate one, for the filaments of the angioblasts can be seen to dip down from the vascular layer to the mesoderm beneath. In the flat living specimen, and in sections which have been made from a specimen which was growing out flat on a cover-slip, there is no intermingling of the mesoderm and the vascular layer, such as is seen in Duval's plate XVI, figure 264. Such an apparent intermingling of the two layers is due to shrinkage. In other words, the angioblasts differentiate out of the mesoderm and form a new layer, which is throughout ventral to the mesoderm. These two plexuses were well known to His, who recognized them in their relations to the cœlom on the one hand and to the angioblasts on the other in his work published in 1868, but described them more fully in the *Lecithoblast und Angioblast* published in 1900. His stated that the two plexuses were at times very hard to analyze.

The plexus of angioblasts is, then, distinguished first by its more ventral position, and secondly by the fact that the cytoplasm of the angioblasts is slightly more granular and reacts slightly more intensely to basic dyes than does the mesoderm. The following criterion, however, is the one which I have found most useful. In the living specimens there seems to be a sort of rhythm in cell division. I have already referred to the fact that the entire endoderm may divide and become so opaque that none of the cells beneath can be seen. At other times the entire plexus of angioblasts over a very extensive zone will pass into the phase of cell division. In this condition the cytoplasm of the plexus of angioblasts becomes very highly refractile and opaque, so that it can be distinguished from the plexus of the cœlom with great ease, even with low powers of the microscope. The protoplasm shows this change for about an hour before the chromosomes pass on to the spindle; so, in order to obtain the nuclear figures characteristic of cell division, one must watch until a few areas in the plexus begin to clear and

then fix the specimen. The time required for the nuclear changes is much less than the time taken for the cytoplasmic changes. According to M. R. Lewis, the nuclear phase lasts about 5 minutes, while the cytoplasmic change takes about an hour. The facts that not every nucleus divides at the same moment and that the cytoplasmic changes have not been recognized explain the failure to note the rhythm of cell division.

Using the criteria for distinguishing angioblasts which have just been indicated, I will now describe what has been made out concerning the vascular system at the stage of 6 somites, both in the living specimen and in sections which have been made from a blastoderm in which the cells had been charted in the total specimen before the sections were cut. For this description the axis of the embryo may be divided into four zones: (1) that part of the head which is covered by the head-fold, as seen from the ventral aspect; (2) the head between the head-fold and the first myotome; (3) the zone of the myotomes; (4) the zone caudal to the myotomes. As has been described, there is a dense band of tissue on either side of the axis of the embryo which divides the area pellucida into an inner dense zone and an outer thinner zone. The area opaqua, on the other hand, is denser along its outer margin. Beginning with the area opaqua, in its outer margin there is a large marginal plexus of vessels partly filled with blood-cells which cling in large masses to the dorsal wall of the vessels. The blood-cells can be distinguished from the angioblasts by the fact that in the edges of the masses they tend to separate from the mass and have a definitely round contour. Angioblasts never have a round contour. In this marginal zone the cœlom is clearly seen, with its dorsal and ventral mesoderm, and the ventral wall of the blood-vessels is very plainly distinguished from the endoderm; but the dorsal wall of the blood-vessels is closely attached to the ventral mesoderm, and in places can not be distinguished from it.

The inner margin of the area opaqua and the outer margin of the area pellucida have two definite plexuses: the dorsal plexus of the cœlom and the scantier ventral plexus of solid angioblasts. Over the dense area on either side of the myotomes the cœlom is no longer in the form of a plexus, but has a complete lumen; for there the body-cavity is well formed. The plexus of angioblasts covering this area is continuous with a plexus of angioblasts along the lateral margin of the myotomes. Caudal to the sixth myotome, the plexus extends for a short distance along the undifferentiated mesoderm, curving a little to the side. Very interesting appearances are to be made out near the first myotome. Extending forward from the lateral border of the first myotome, the chain of angioblasts representing the aorta can be seen up to the margin of the head-fold, when it disappears under the fold. Opposite the first myotome, and extending forward from its mesial border, there is also a chain of angioblasts along the hindbrain, and this chain of angioblasts connects with the aorta above the first and between the first and second myotomes. The chain of cells along the margin of the hindbrain I should not recognize as angioblasts in sections; but in the living blastoderm they have exactly the appearance of the angioblasts of the aorta and connect with them by slender filaments.

In the region of the head, which can not be analyzed in the living blastoderm, the angioblasts representing the heart are well known and easily identified. The two cardiac primordia have met in the mid-ventral line and can be followed a short distance into a ventral cephalic aorta, which gradually becomes too indefinite for recognition. The dorsal cephalic aorta is very clear opposite the region of the heart, gradually disappearing farther forward. Thus, within the embryo there are chains of angioblasts representing the heart, most of the ventral cephalic aortæ, and a part of the dorsal cephalic aortæ. Opposite the region of the heart the two dorsal aortæ are definite, tiny vessels which emerge from under the head-fold and are continued partly as a plexus of solid angioblasts and partly as a vessel along the ventro-lateral border of the myotomes. The entire plexus which is exposed on the ventral aspect connects with the plexus of angioblasts of the area pellucida. In this account I wish to emphasize the very early appearance of angioblasts along the hindbrain—the forerunner of the so-called vena capitis medialis, which I prefer to call the primitive vessel of the hindbrain. I have not yet a sufficient number of observations to prove, first, whether the transitory vessel of the hindbrain does differentiate *in situ* while the aorta is differentiating, and secondly, whether it is established earlier than the vessels of the forebrain; but both of these propositions seem to me to be very probable.

In this study I have found Williams's (1910) very careful description of the vascular system of the early chick embryo of great value. His specimen of 6 somites is clearly a little farther advanced than mine. He found that at 6 somites the aortæ were established, but were still small and irregular. He then observed a vessel along the neural tube (hindbrain) connected with the aorta in the first and second interspaces, the vessel in the first interspace being nearly as large as the aorta itself.

It is now important to consider how the plexus of angioblasts increases. This occurs first by cell division and secondly by the differentiation of new angioblasts. Cell division in the plexus of angioblasts is very extensive, for in watching the living specimens it is seen that large areas of the plexus divide at the same time, and in these cycles of cell division every cell of the plexus divides. Besides this very extensive cell division new angioblasts differentiate and join the plexus. This process can best be observed along the mesial border of the dorsal aorta itself, near the lowest myotome. Here practically every blastoderm between 6 to 10 somites will show one or two isolated angioblasts which are very readily marked from the dense mesoderm beneath. Out in the zone of the developing cœlom the distinction is by no means so easy. These angioblasts are either single, spindle-shaped cells or clumps of two or three cells. When observed they are seen to put out tiny filaments toward the wall of the aorta, which at once responds by putting out a filament toward the young cells. These tiny filaments meet halfway, and the new angioblasts thus join the wall of the aorta. They gradually approach the wall and become incorporated into the vessel. As the new cells become a part of the wall their protoplasm becomes less granular and they acquire a lumen. The exact process by which angioblasts acquire a lumen is extremely difficult to determine, and concerning this point nothing has yet been added to the original description of His.

These observations on the origin of the aorta, as well as the observations indicating that the transitory vessel of the hindbrain differentiates from angioblasts *in situ*, at once lead to the general question of the origin of the vascular system All are agreed—on the foundation of the work of von Baer, Remak, and His—that certain cells of the embryo differentiate to form angioblasts or vasoformative cells in the early stages of embryonic life, and that these angioblasts increase by cell division. There has, however, been a wide divergence of opinion as to whether the differentiation of new angioblasts continues throughout life or whether there is a limit to the period of differentiation, after which all the new angioblasts must come from the growth of preceding endothelium.

It is in relation to these two theories that I am making these studies on the living blastoderm. It is, I think, clear that the study of blood-vessels in the stages of their differentiation does not prove that they continue to differentiate out of mesoderm throughout life, any more than the finding of several primordia for the thymus proves that new thymus glands continue to arise throughout life. The question of the origin of the blood-vessels is now an exact one—namely, which vessels arise in the embryo (as does the aorta, at least, in part) by differentiation of angioblasts, and which grow from previous vessels. In other words, how long does the period of differentiation of angioblasts continue?

His formulated the theory that the embryo itself is invaded by angioblasts from the yolk-sac. This theory was based on the following observations: First, that along the myotomes in the early stages angioblasts can be seen streaming toward the axis of the embryo from the outer margin of the area pellucida; second, that he observed no such streaming of angioblasts toward the axis of the embryo in the zone between the head-fold and the first myotome (here, as a matter of fact, a few angioblasts can be found in early stages, but are much scantier in number than lower down); and third, that the most cephalic part of the head does not receive angioblasts from the membranes. From these observations he concluded that the vessels of the axis of the embryo must arise from a growth of the angioblasts which could be seen to enter the embryo at certain places.

Although these observations of His are for the most part correct, that a differentiation of new angioblasts does take place along the axis of the embryo was shown by two series of experiments. First, those of Hahn, who cut out the membranes of one side of a chick in the stage of the primitive streak and obtained a few specimens in which the membranes were entirely lacking, but the aorta was formed on the injured side. Second, the experiments of Reagan, in which he cut off a part of the head of the chick in the stages just before and just after the head-fold is visible, and allowed the isolated parts to remain in the egg and develop. In these isolated fragments he obtained vessels.

The fact that angioblasts do differentiate in the axis of the embryo is conclusively proved by my observations, having watched certain cells differentiate and join the aorta in the living blastoderm. In what I have called the second zone of the axis of the embryo—that is, the zone between the head-fold and the first myotome—the process can not be followed with such minute detail as is

possible opposite the myotomes, because in the former case the heart lifts this zone of the embryo from the cover-slip; but every specimen shows chains of angioblasts which are apparently differentiating *in situ* in this area. None of the experiments or observations here recorded take into account the ultimate point of origin of the cells which differentiate into angioblasts.

By the time the chick has 9 somites the dorsal aorta is readily seen behind the head-fold as a complete vessel if the living embryo be viewed from the ventral aspect. Opposite the upper myotomes the aorta is directly ventral to the myotomes, but it gradually curves outward, so that opposite the ninth myotome and the undifferentiated mesenchyme it lies along the lateral border of the myotomes. Along its entire lateral border it is connected with the plexus of the area pellucida. As has been shown by Evans, the entire caudal portion of the aorta is a part of the capillary plexus of the area pellucida. In summing up the question of the origin of the aorta it may be said that it differentiates as a part of the plexus of angioblasts, extending over the entire area vasculosa, and is increased by the addition of new angioblasts along the axial line of the embryo.

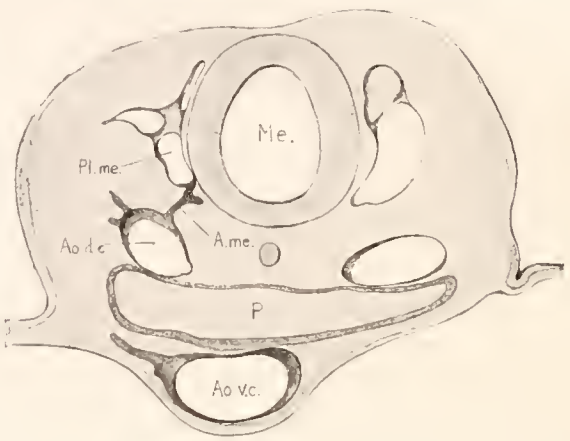


FIG. 1.—Transverse section of an injected chick of 12 somites, passing through the middle of the mesencephalon, to show the vascular plexus on the mesencephalon. On the left, the dotted area shows how far the ink passed through a dorsal artery from the aorta into the plexus on the midbrain. The section is from the same series as those in figures 2 and 3, and it is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The section is 50$\mu$ thick and is unstained. ×133. *A. me.*, artery to the plexus on the mesencephalon; *Ao. d. c.*, aorta dorsalis cephalica; *Ao. v. c.*, aorta ventralis cephalica; *Me.*, mesencephalon; *P*, pharynx; *Pl. me.*, plexus on the mesencephalon.

By the time the chick has 9 somites the aorta can be injected; it forms from the plexus of angioblasts while the seventh, eighth, and ninth somites are forming. While the dorsal aorta of the region of the myotomes is best seen in the living chick, the cephalic aorta is best observed in an injection. As can be seen in plate 1, figure 3, the heart is a simple tube. In some specimens, even with 10 somites, it is in the exact mid-ventral line; in others, as in plate 1, figure 3, it is slightly to the right. In some of my injections the ventral aorta has numerous mesial and lateral sprouts; in this particular specimen these sprouts are more numerous along the dorsal cephalic aorta.

In one of my injections the heart itself shows a little of the primitive plexus. The dorsal cephalic aorta shown in plate 1, figure 3, is still in the form of a plexus; from the arch of the aorta two very constant sprouts extend to the ventral surface of the forebrain. The development of these sprouts is well shown in a figure by Evans from a duck embryo with 13 somites (fig. 398) in the "Manual of Human Embryology" (Keibel and Mall).

The mesial sprouts do not form permanent vessels; but in one very interesting abnormal embryo which I injected these mesial sprouts had formed anastomoses across the mid-line. They are thus, in this specimen, analogous to the vessels

which cause the fusing of the two aortæ lower down. Opposite the region of the heart some of the lateral sprouts extend out in the somatopleure, as shown in plate 2, figure 1, and in sections in Duval's plate XVII, figure 276. Opposite the midbrain some of these lateral sprouts may connect with the superficial plexus.

The dorsal cephalic aorta itself, as seen in plate 1, figure 3, is very large. From the dorsal aspect it is broad and flat and is placed in a nearly exact transverse axis instead of in the oblique position which it subsequently assumes.

The next stages in development, including the relations of the primitive cerebral vessels and the cardinal system of veins up to the stage of 14 somites, I shall describe with the aid of two total preparations from chicks of 12 and 14 somites and three sections from the stage of 12 somites (figure 2 of plate 1, figure 1 of plate 2, and text-figs. 1, 2, and 3).

At the stage of 12 somites the aorta is very readily injected. The vessels to the brain, however, though they connect with the aorta, are difficult to inject. In plate 1, figure 2, is shown the usual result of injecting a small quantity of ink into the omphalo-mesenteric veins at the stage of 12 somites; the ink passes through the heart and the aorta into the capillaries, which are the forerunners of the omphalo-mesenteric arteries. This is true, even though vessels to the entire brain—that is, to the forebrain, midbrain (text-fig. 1), and hindbrain (text-fig. 2)—are present and connect with the aorta, although the common cardinal vein is present down to the twelfth interspace (text-fig. 3) and the entire lateral border of the aorta opposite the myotomes is connected with the plexus of the area vasculosa. In plate 1, figure 2, the only branch of the aorta injected is an unusual dorsal branch opposite the tenth somite, passing out into the somatopleure. In order to fill these different branches of the aorta in the stages shown in figure 2 of plate 1 and figure 1 of plate 2 before the circulation has begun, it is necessary to introduce the needle into the aorta and inject, as it were, backwards. In this way the pressure in the aorta is probably raised, the heart being sufficiently stimulated by the ink to force the injection mass into the tiny channels that would otherwise remain empty. Indeed, after the circulation has begun, if only a very small quantity of ink enters the heart it will return to the area vasculosa without injecting the branches of the aorta within the embryo. These fill up only as the injection is continued and the heart becomes well filled with ink.

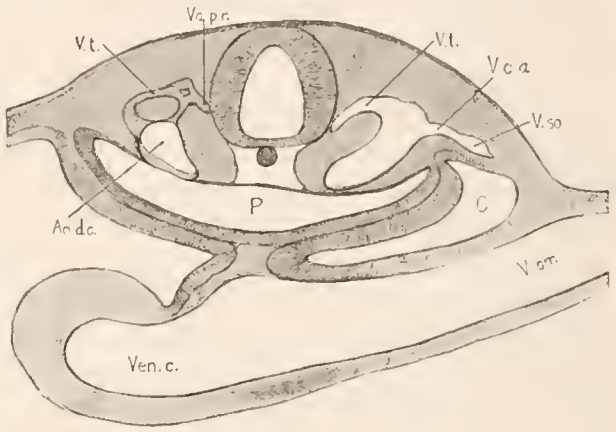


FIG. 2.—Transverse section of an injected chick of 12 somites, passing through the first interspace to show the relations of the primitive vessel of the hindbrain, the transverse vein of the first interspace, and the anterior cardinal vein. The section is from the same series as those of figures 1 and 3, and is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The section is 50 $\mu$ thick and is unstained. ×133. *Ao. d. c.*, aorta dorsalis cephalica; *C*, cœlom; *P*, pharnyx; *V. c. a.*, v. cardinalis anterior; *V. om.*, v. omphalo-mesenterica; *V. so.*, vein of the somatopleure; *V. t.*, v. transversa of the first interspace; *Va. p. r.*, vasa primitiva rhombencephali; *Ven. c.*, ventriculus cordis.

That there are vessels within the embryo at the stage of 12 somites which can be injected from the aorta is proved by three sections from an injected chick of this stage (text-figs. 1 to 3). These sections are best followed by comparing them with the specimen shown in plate 2, figure 1, from a chick with 14 somites. The section shown in text-figure 1 passes through the midbrain and shows a plexus of vessels on the midbrain fully as large as the aorta itself. On the left side of the section (right side of the embryo) is a slender artery containing ink, connecting this plexus with the aorta. This plexus of large vessels on the midbrain, shown in text-figure 1, also connects with a single longitudinal vessel along the hindbrain at this stage.

These neural vessels, which at this stage are connected with the aorta, have no vent, which probably explains the great difficulty in injecting them. They are full of fluid, and though the ink enters them from the aorta, it does not penetrate far (text-fig. 1). This point is, I think, interesting in connection with the time of the beginning of circulation. As is well known, the heart begins to beat early in the second day. I have made a number of observations which show that it beats at the stage of 10 somites. In one instance I injected an embryo of 10 somites in which the heart was not beating, and when a small amount of the ink entered the heart it was stimulated to beat. In another instance I had been watching an isolated blastoderm of 9 somites for over an hour when the heart began to beat. This occurred just as the tenth somite was beginning to appear. It is therefore quite certain that the stage of 10 somites marks the beginning of the heart-beat.

FIG. 3.—Transverse section of an injected chick of 12 somites passing through the twelfth interspace to show the relation of the posterior cardinal veins to the aorta. The section is from the same series as figures 1 and 2, and is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The section is 50 $\mu$ thick and is unstained. ×133. *Ao.*, aorta; *N*, nephrotome; *V. c. p.*, v. cardinalis posterior.

At the time the heart begins to beat its venous end connects with the extensive capillary plexus of the area pellucida in which the omphalo-mesenteric veins arise, and the entire aorta opposite the myotomes is connected with the capillary plexus in which the omphalo-mesenteric arteries arise. In other words, there is a plexus of vessels covering the entire area opaqua and area pellucida which connects with the venous end of the heart and with the entire dorsal aorta of the embryo opposite the zone of the myotomes. In the area pellucida this plexus of vessels is filled with fluid, but there are very few free cells in the vessels. After the heart begins to beat most of the isolated blastoderms show occasional wandering cells of various types that float into the vessels of the area pellucida, showing that these vessels are full of fluid; and when one of these cells approaches the heart in the omphalo-mesenteric veins it oscillates back and forth with each beat. It is thus very strikingly apparent that the circulation does not begin for a considerable time after the heart begins to beat. It is difficult to note the exact time of the beginning of the circulation while the chick is on the yolk, for the few red blood-corpuscles that are forced into the aorta are inconspicuous with the powers of the microscope that can be used. In the isolated blastoderms the earliest chick in which I have seen the circulation begin was one of 17 somites. At the beginning of circulation a few corpuscles are shot into the aorta with each

beat of the heart. The mounting of the blastoderm on a cover-slip, however, interferes with the circulation much more than with the heart-beat, because the flattening of the blastoderm tends to flatten the vessels and thereby impede the circulation. This is often strikingly shown when through mechanical difficulties the circulation is entirely cut off on one side of an isolated blastoderm and not on the other. It is therefore probable that the circulation begins in the chick about at the stage of 15 or 16 somites. It is interesting to note that it is at this stage that the duct of Cuvier breaks through into the omphalo-mesenteric veins, whereby the dorsal aorta and the veins of the embryo become connected with the venous end of the heart.

It is thus clear that at the stage of 12 somites, when the head of the embryo contains a complete aorta and a neural system of vessels which consists of a plexus of large vessels on the forebrain and midbrain, and a single channel on the hindbrain, there is no circulation through these vessels due to the beat of the heart.

The connections of the vessels of the brain with the aorta are of importance. The arteries connecting the vessels of the forebrain with the aorta consist of a group of vessels just at the primitive arch of the aorta. These are shown in plate 2, figure 1, and have been thoroughly demonstrated by Evans. These arteries connect with the neural vessels at the base of the optic cup, in the groove representing the line between the telencephalon and the diencephalon. Subsequently this group of vessels divides into two arteries, one of which encircles the optic stalk and the other extends caudalward along the ventral border of the thalamus and the midbrain (plate 6). Opposite the midbrain there is a group of tiny arteries connecting the plexus with the aorta, one of which is shown injected in a chick of 12 somites in text-figure 1.

It is very clear (in the section of text-figure 1) that the vessels to the neural plexus are direct, dorsal branches of the aorta. The vessel along the hindbrain connects with the aorta by two groups of tiny branches, one cephalic and the other caudal to the otic vesicle. These branches are also for the most part direct dorsal branches. In one of my sections, however, two arteries to the vessel of the hindbrain are placed with reference to the aorta, as are the vessels on the left side in text-figure 2—that is, one is dorsal and the other dorso lateral. These connections between the aorta and the primitive vessel of the hindbrain are shown, injected, by Evans, in his figure 393 in the "Manual of Human Embryology" (Keibel and Mall).

As far as the vessels which connect the vascular channel of the hindbrain with the aorta are concerned, it has been shown that they differentiate as angioblasts at the stage of 6 somites, while the aorta and the neural vessels are differentiating. The origin of the primary plexus of deep vessels on the surface of the forebrain and midbrain requires more careful study during the stages of from 6 to 12 somites. It is probable that these vessels differentiate, and that their connections with the aorta differentiate, as does the preliminary vascular channel of the hindbrain. The development of the deep neural vessels and the origin of the superficial plexus of vessels opposite the brain, as well as the origin of the primary head-vein, will be taken up subsequently.

## ORIGIN OF THE CARDINAL VEINS.

It is now important to consider the cardinal veins—how they arise, how they become related to the primitive neural vessels, and how they become connected with the heart through the duct of Cuvier. The general relations of the cardinal veins are best shown in plate 2, figure 1, from a chick of 14 somites, but their origin can be traced back to the stage of 9 somites. They form as a longitudinal anastomosis which connects diverticula of the aorta that project dorsalward between the somites. In 1906 these dorsal diverticula were described by Grafe, who stated that the cardinal veins arose from sinus-like projections from the aorta. That the cardinal veins arise from dorsal intersegmental branches of the aorta was shown by Rabl in 1892 and by Hoffmann in selachians in 1893.

The condition of the aorta just before the diverticula arise is of importance. Up to the stage of 9 somites it is clear that the entire aorta which can be seen from the ventral aspect in the living blastoderm is connected with the plexus of the area vasculosa through so-called ventral branches which extend lateralward. Even cephalic to the first myotome a few chains of angioblasts connect the aorta with the plexus of the area vasculosa. However, these tiny branches all along the lateral border of the aorta are seldom injected, except opposite the caudal end of the aorta (plate 1, fig. 2).

When the chick has 9 somites a new set of aortic branches begins to form, which are very distinct from the lateral vessels. In the living blastoderm of from 9 to 12 somites it can be seen that diverticula of the aorta project dorsalward into the interspaces. The more cephalic of these diverticula are dorso-lateral, as shown on the right side in text-figure 2, from a section through the first interspace; the more caudal ones are distinctly dorsal, as seen for the twelfth interspace in text-figure 3. This is due to the fact that at the stage of 12 somites the aorta is obliquely placed with reference to the lateral margin of the myotomes. As shown in text-figure 2, in the first interspace the lateral margin of the aorta is in the lateral line, while in the twelfth interspace, as shown in text-figure 3, the aorta is directly under the lateral line. The first two of these diverticula have been seen at the stage of 9 somites; and they are present in all of the interspaces at the stage of 12 somites. In a total preparation of a chick of 12 somites the ink lodges in these dorsal diverticula and forms dark streaks across the aorta from the dorsal aspect; these streaks are very characteristic, but are difficult to indicate in a drawing. The specimen of plate 1, figure 2, shows such streaks across the aorta in the interspaces.

The diverticula begin at the time when the first two somites lie within the arch formed by the two omphalo-mesenteric veins where they join the heart. In this connection I have tried to determine whether there is a constant relation in regard to the time when the cardiac or head fold reaches the level of the first somite; and in this regard the figures in His's "Untersuchungen ueber die erste Anlage des Wirbelthierleibes" (1868), plate XII, and those in Lilly's "Development of the Chick" (1908) are the most helpful. In general, at the stage of 9 somites the position of the first somite is about as shown in Lilly's figure 61, page

106; but I have chicks of 10 somites in which the first somite is farther from the head-fold than in the usual specimen of 9 somites. As a rule the head-fold is along the cephalic border of the first somite when the embryo has 12 somites; but in some specimens, such as in His's plate XII, figure 20, there is at this stage an interval between the first somite and the head-fold. After the cephalic curve of the midbrain has formed, as shown in plate 2, figure 2, the embryos are not as flat from the direct dorsal aspect and the point can not be tested with the same definiteness.

The longitudinal vessel which connects these diverticula in the lateral line of the embryo is the common cardinal vein (plate 2, fig. 1). The cardinal vein has two fundamental relations—on the one hand to the primitive vascular channel of the hindbrain and on the other hand to the venous end of the heart. As is shown in plate 2, figure 1, and in the section in text-figure 2, the cardinal vein becomes connected with the neural vessel by two cross-anastomoses in the first and second interspaces. Of these vessels the one in the first interspace is the larger and more important. The cardinal vein itself is not shown on the left side in text-figure 2 (right side of the embryo), since the transverse vein of the first interspace is slightly oblique, as is plainly seen in plate 2, figure 1.

The transverse vein of the first interspace has been described and illustrated by Evans; and has been traced back to the stage of 6 somites by Williams. In the chick it is an important channel in the second and third days of incubation, for it is the channel by which all of the blood for the brain drains into the cardinal vein and thence to the heart. The transverse vein of the first interspace is characteristic of the chick. It does not form in the pig where the transitory vessel of the hindbrain connects with the cardinal vein in front of the first somite instead of in the first interspace.

At the stage of 12 somites the dorsal diverticula of the aorta are present in all the interspaces, but there is not yet a continuous vein connecting them opposite the lower interspaces. The cardinal veins begin to form at a very early stage, when the zone along which they form is close to the aorta (text-fig. 3), so that the primitive common cardinal vein is an accompanying vein to the aorta. It is this accompanying vein of the aorta which connects with the venous end of the heart, forming the duct of Cuvier. So close is its relation to the aorta that the duct of Cuvier may be regarded as a direct connection between the dorsal aorta and the omphalo-mesenteric veins.

The position of the duct of Cuvier is well known, and is shown in plate 2, figure 1. At the stage of 14 somites, as shown in this figure, the common cardinal vein opposite the second, third, and fourth somites is in the form of a plexus; and it will be noted that there is a vessel extending lateralwards from this plexus opposite the cephalic border of the omphalo-mesenteric veins, and a similar vessel opposite the caudal border of the vein. These two vessels are in the somatopleure dorsal to the omphalo-mesenteric veins. This is very clearly shown in the section in text-figure 2. The more cephalic of these two vessels (*V. so.*) develops, as I shall show for the pig, into veins which drain the body-wall over

the region of the heart cephalic to the duct of Cuvier. They receive their blood from lateral branches of the aorta (of which the lateral artery opposite the heart, shown in plate 2, fig. 1, may be one) and are analogous to the branches of the umbilical veins below the duct of Cuvier.

Of the veins of the somatopleure, those which are opposite the caudal border of the omphalo-mesenteric veins join the omphalo-mesenteric vein in the septum transversum of His, as shown on the right side of text-figure 2. The connection, which has not taken place in the specimen in plate 2, figure 1, at the stage of 14 somites, occurs at the stage of 15 somites, as was shown by Evans. In making injections at the stage of 15 or 16 somites it sometimes happens that the ink first injected does not fill the neural vessels, but runs from the aorta into the duct of Cuvier through direct aortic branches, such as that shown in the third interspace in plate 2, figure 2.

One of the most interesting points in connection with the duct of Cuvier is that it forms just about the time or just before the time when the circulation begins, which is probably of great importance from the standpoint of the physiology of the embryo. Thus plate 1, figures 2 and 3, and plate 2, figure 1, represent the blood-vessels of the chick before the circulation has begun, while figure 2 of plate 2 and figure 1 of plate 3 represent a series of chicks in which the circulation has commenced. Inasmuch as the duct of Cuvier has not connected with the omphalo-mesenteric veins (sinus venosus) at the stage shown in plate 2, figure 1, the longitudinal plexus and vessel of the lateral line at this stage is a common cardinal vein which will be divided into an anterior and a posterior division by the position of the duct of Cuvier. From a comparison of figures 1 and 2 of plate 2, figure 1 of plate 3, and plate 6 it is clear that the anterior cardinal vein must increase in length at the expense of the posterior cardinal vein as the heart shifts caudalward. In these figures it is plainly shown that the cardinal system opposite the duct of Cuvier continues in the form of an extensive plexus (see also plate 1, fig. 1, of the pig) and that the plexus ultimately gives rise to the umbilical veins.

This completes the general account of the blood-vessels of the chick before the circulation has begun; that is, up to the stage of plate 2, figure 1. I shall take up, under two headings, the study of the further development of the primitive blood-vessels in the stages in which the blood is circulating; first, the vessels of the brain and their relation to the primary head-vein; second, the vessels of the spinal cord. The primitive vessels of the nephrotomes will be taken up in connection with the pig embryos. It is of course evident that the two divisions overlap, for the vessels of the brain begin in the period before the circulation commences.

## VASCULAR SYSTEM OF THE BRAIN AND THE PRIMARY HEAD-VEIN.

As has been shown, the neural vessels begin to form very early, before there is any circulation, and indeed before the heart has begun to beat. The primitive vessel of the hindbrain differentiates at the stage of 6 somites as a chain of angioblasts along the border of the hindbrain, and at the time it is differentiating

connects with the aorta by chains of angioblasts which are forerunners of direct dorsal branches of the aorta.

Exactly when the angioblasts along the forebrain and the midbrain can be identified has not been determined, but at the stage of 12 somites there is a plexus of large vessels along the lateral surface of the forebrain and midbrain extending to the ventral surface of the forebrain at the base of the optic vesicle and anastomosing with the primary vascular channel of the hindbrain. This plexus connects with the aorta just at the base of the optic vesicle, as was shown by Evans in his figure 398 for a duck embryo of 13 somites in the "Manual of Human Embryology" (Keibel and Mall). At the stage of 12 somites this plexus also connects with the aorta opposite the midbrain, as shown in text-figure 1.

This deep primary plexus, which I have uniformly represented in a gray tone, soon gives rise to a superficial plexus opposite the region of the forebrain and the midbrain, as shown in text-figure 1. In this superficial plexus there develops a venous channel for the forebrain and the midbrain, as will be seen in plate 2, figure 2, from a chick of 16 somites, which is the stage when the blood begins to circulate. The superficial plexus opposite the forebrain and the midbrain arises, for the most part, from the deep plexus (text-fig. 1), but I have also injected a few tiny connections between the superficial plexus and the aorta itself in early stages. These, however, disappear and the superficial plexus drains only the deep plexus.

The vein which develops within the superficial plexus is characteristically placed, and is very adequately shown by Evans for the stages of 17 to 25 somites (Anatomical Record, 1909, III, figs. 3 to 6). At the stage of 29 somites this primitive cerebral vein is clearly shown in plate 6. Owing to the flexure of the midbrain, the primitive cerebral vein (*v. cap. p. 1*) runs directly across the thalamus; and it receives a very interesting series of branches. It is obvious that a very large number of the primitive veins opposite the cerebrum drain the eye. Beginning with the position of the Gasserian ganglion, as seen in plate 6, there is a plexus of veins which I have called the primitive maxillary veins (*v. m. p.*), which drain the inferior part of the eye and the most anterior border of the cerebrum. These veins have usually been called the primitive inferior ophthalmic veins, and, according to the function which they actually perform at the stage of plate 6, this would be perhaps a more logical name. However, the stage when this plexus drains mainly the eye is very transitory. Soon the capillaries of the maxillary arch develop and the plexus of veins which, at the stage of plate 6, clearly lies in the maxillary arch, drains all of the structures of that arch, the roof of the mouth, and the nose. The position of the maxillary vein and its corresponding artery in the maxilla is shown for the pig in plate 7. In the chick of the fourth and fifth days of incubation this group of veins clearly drains the entire maxilla and receives branches from the most anterior part of the cerebrum and a group of inferior ophthalmic veins, of which one of the most important runs in the optic stalk. Therefore I have preferred to limit the name primitive inferior ophthalmic veins to the branches of the primitive maxillary vein instead of calling

the entire trunk the ophthalmic veins. The emphasis on the fact that this group of veins belongs in the maxilla, bringing it into line with the veins of the mandibular arch and with the veins from the rest of the aortic arches, is interesting in connection with the origin of the middle segment of the primary head-vein.

Cephalic to this plexus of maxillary veins is an extensive series of veins from the marginal vein of the optic cup. The vein in the margin of the optic cup is very characteristic. Above these is a smaller, but very important, group of veins which drain the cerebrum proper. As can be seen in plate 6, they tap the deep plexus of the cerebrum at their tips; they gradually creep dorsalward on the deep plexus until they meet with those of the opposite side in the mid-dorsal line. The anastomosis of these veins in the mid-dorsal line will ultimately give rise to the superior sagittal sinus, as has been shown by Mall and Streeter. On account of the relation of the primitive veins of the neural tube to the ultimate formation of the dural sinuses, this process of the creeping of the primitive veins toward the mid-dorsal line on the deep plexus is very important.

Over the thalamus at this stage is the main root of the primitive cerebral vein and one large accessory root. For the midbrain the superficial branches of the primitive cerebral brain have not yet appeared, and all the blood of the midbrain drains through the deep plexus toward a characteristic deep vessel along the cerebellar ridge, which joins the primitive vessel of the hindbrain. In plate 2, figure 2, it is clearly shown that at the time when the circulation begins all the venous blood of the forebrain and midbrain must pass through the deep channel of the hindbrain and the transverse vein of the first interspace in order to reach the heart. This figure (plate 2, fig. 2) shows that the vessel of the hindbrain is mesial in position both to the primitive cerebral vein and to the anterior cardinal vein. Plate 6 shows that it is also farther dorsal than either of these veins; and also, what is well known, that the primitive vessel of the hindbrain is mesial to the Gasserian ganglion, the acoustic complex, the otic vesicle, and the ganglion of the glosso-pharyngeus. The cephalic end of the ganglion of the vagus, on the other hand, is mesial to the transverse vein of the first interspace; that is, the primitive vessel of the hindbrain runs down to the region of the cephalic end of the ganglion of the vagus.

The primitive vessel of the hind-brain serves as a transitory vein for the brain of the chick during the second and third days of incubation, as is very evident in any living chick. On the other hand, it serves as the only channel for the blood to the hindbrain, and it can receive arterial blood directly from the aorta through tiny branches. These branches are so small and are so seldom fully injected that it is probable that only a small amount of blood actually passes through them in the living chick into the primary channel of the hindbrain. The permanent arteries for the hindbrain develop later, as will be shown.

Plate 6 shows how the primary vascular channel of the hindbrain ceases to serve as a vein for the forebrain and midbrain, and how the true head-vein, the vena capitis prima, develops. The specimen here shown also indicates the fate of the primary vessel of the hindbrain. The deep plexus of the cerebrum, the

thalamus, and the midbrain has now made an almost complete covering for that part of the neural tube. Over the midbrain the plexus is farthest developed and has anastomosed across the mid-dorsal line with the plexus of the opposite side; over the thalamus and the cerebrum the deep plexus has almost reached the mid-line. The primary artery of the brain which supplies this extensive plexus divides into two branches—first, into a large arterial plexus which curves around the dorsal margin of the optic stalk and leads to the plexus around the eye and to the plexus of the cerebrum, and partly supplies the plexus of the thalamus; second, into an artery which curves along the ventro-lateral border of the thalamus and the midbrain, and is approaching the hindbrain. This artery will soon meet the ascending artery seen along the ventro-lateral border of the rhombencephalon.

Opposite the hindbrain the development of the vessels, both the arteries and the veins, is most interesting. As is shown in plate 6, there is now a most important new vein. This is as yet a tiny, irregular vessel, hardly larger than a capillary, which connects the veins of the maxillary, the mandibular, and the second aortic arch with the anterior cardinal vein. The primitive vessel of the hindbrain is a vein for the brain only; this new capillary develops out of the capillaries of the visceral arches and by means of the relation of the maxillary veins to the primitive cerebral vein it receives the blood of the primitive cerebral vein and hence it becomes a true head-vein. We shall call this new vein, which is usually called the vena capitis lateralis, the middle segment of the vena capitis prima (*v. cap. p. 2*), and will say that as soon as this anastomosis between the primitive maxillary veins and the anterior cardinal veins takes place we can speak of a primary head-vein which extends from the region of the thalamus to the duct of Cuvier and drains the structures of the head, namely, the brain and the tissues of the visceral arches.

The specimen in the drawing of plate 6 is not shown from an exactly lateral aspect, but is tilted slightly to show the ventro-lateral surface of the hindbrain; but even with this tilting it is clear that the general position of the superficial vessel is such that it can become a direct line between the primitive cerebral vein and the anterior cardinal vein. This direct line is very plain in plate 2, figure 2. In other words, it is a more favorable vessel for the drainage of the large primitive cerebral vein than is the primitive vessel along the hindbrain.

The exact course of this tiny chain of new capillaries is most interesting, because it conforms so closely to the structures that are present before it develops. In this connection the relation of this new capillary to the Gasserian ganglion is important to note, because it has been so little understood. As is well known, the ganglion arises from the wall of the pons at the point shown in plate 6, grows ventralward, and becomes adherent to the skin, making the placode of the trigeminus. If sections from specimens at the stage of plate 6 are studied, it will be seen that it is this attachment of the Gasserian ganglion to the skin, occurring at the stage when the tiny capillaries that give rise to this superficial vein begin, that renders it impossible for the new capillaries to pass lateral to the ganglion; hence they grow mesial to it. The primitive vessel of the hindbrain is mesial

to the Gasserian ganglion, but lies against the hindbrain; the primary head-vein is also mesial to the ganglion, but lies ventral to the hindbrain.

On the other hand, the new capillaries pass dorsal to the placode of the acoustic complex, and the slight dorsal curve of the primary head-vein (which is very evident in plate 6) indicates this adjustment of the vein. The placode of the acoustic complex is indicated in plate 6 by a film over the primitive vessel of the hindbrain opposite the root of the eighth nerves. The vena capitis prima passes ventro-lateral to the otic vesicle and again curves slightly dorsalward opposite the ganglion of the glosso-pharyngeus.

In another injected specimen of this stage the superficial vein is a slender capillary plexus spanning the gap between the second aortic arch and the anterior cardinal vein, and not yet connecting with the veins of the maxillary arch. Thus this middle segment of the vena capitis prima (the so-called vena capitis lateralis) begins as an irregular capillary plexus between the aortic arches and the anterior cardinal vein. It becomes a true head-vein, in the sense that it drains the entire head, whereas the primitive vascular channel of the hindbrain (vena capitis medialis) is a true neural vessel draining the brain only and not the entire head.

Up to the stage when the capillaries of the visceral arches develop, the primitive channel of the hindbrain serves as the only drainage channel in the head, and this means practically for the brain alone; but as more structures in the head differentiate, a new vascular channel develops to drain these structures. This new chain of capillaries which receives the blood of the primitive cerebral vein by means of the relations of the maxillary veins is so direct and so favorable a channel for the blood of the primitive cerebral vein that the vena capitis prima develops very rapidly at the expense of the primitive vessel along the hindbrain.

By far the most interesting way to follow this transformation is by watching the living chick. As is very clearly shown in plate 6, there is a stage when there are two venous channels for the head of the embryo—a large, deep channel along the hindbrain and a superficial tiny capillary chain farther ventral and farther lateral. While this more lateral channel is very tiny, it is hard to see it in the living chick, because there are few if any blood-corpuscles in it, and it is by the injection of blood, as it were, that one sees the vessels. In one chick opened toward the close of the third day and kept in a warm box, the two veins were of equal size when first observed, but in the course of about 2 hours the ventral channel had become by far the larger. This important change can be followed in the living chick either by opening a number of eggs at the close of the third day of incubation and observing the veins by the blood within them or by keeping a single chick of the right stage under observation for 4 or 5 hours.

In such living specimens it can be seen that the deep vessel of the hindbrain, which remains as a single vessel for 2 days, becomes a capillary plexus as soon as the mass of venous blood from the forebrain and midbrain becomes shunted through the superficial vein. In an injection many interesting details of this process can be made out which are not so clearly seen in the living chick. In a stage still earlier than that shown in plate 6, the deep vessel of the hindbrain

begins to show very characteristic dorsal branches which conform to the surface of the hindbrain and to the roots of its nerves. In fact, the first of these branches, as can be seen in plate 6, tend to surround the root of the trigeminus, the root of the eighth nerves, and the otic vesicle. While these branches of the primitive vessel of the hindbrain are forming, the vessel itself also becomes a plexus. I have injections which show how this takes place. At first the single channel gives rise to a plexus of very large vessels which tend to run longitudinally, following the pattern of the original vessel. Gradually the vessels of this plexus become smaller and the longitudinal pattern is lost. I have not illustrated the development of the plexus on the hindbrain for the chick, but this point is well shown for the pig in plate 7, and the principles are the same in both forms. The plexus on the hindbrain ultimately covers the hindbrain as completely as the plexus on the midbrain shown in plate 6, but the pattern of the plexus is modified by the structures of the hindbrain: (1) by the roots of the nerves and their sensory ganglia; (2) by the otic vesicle, which for a time lies close to the hindbrain; (3) by the special vascular structure of the roof of the fourth ventricle. As has been said, the plexus into which the primary vessel of the hindbrain first breaks up tends to have a longitudinal pattern; the ultimate plexus over the hindbrain, on the other hand, tends, like the rest of the neural plexus, to show indistinct transverse lines. This is, I think, plain in plate 7, and it leads to the subject of the new arterial supply for the vessels of the hindbrain.

A most important point in the history of the transformation of the primitive vessel of the hindbrain concerns its relation to the neural arteries, and this point is well brought out in plate 6. Taking into consideration the entire neural tube, it is originally supplied by a series of arteries from the aorta: (1) a group of vessels to the forebrain, that is, to the cerebrum and the thalamus, at the base of the optic vesicle from the primitive arch of the aorta; (2) a few small arteries opposite the midbrain; (3) a series of small arteries to the primitive vessel of the hindbrain; (4) a series of intersegmental arteries, of which the most cephalic is in the first interspace. In plate 6 an artery is shown on the right side from the primary arch of the aorta, which is growing along the ventro-lateral surface of the thalamus and the midbrain, and this artery is approaching a new artery, which is at the same time growing forward along the hindbrain. This new artery is very important; it starts as a longitudinal anastomosis along the neural tube between the segmental arteries. In plate 6 it connects the first, second, and third segmental arteries, which are occipital vessels, and is growing forward, making more and more new connections with the deep vessel of the hindbrain. Plate 6 shows none of the primitive arteries which connect the primitive vessel of the hindbrain directly with the aorta; but in plate 7, from a pig of a still older stage, it is very interesting to note that two of these original arteries still persist and take part in the formation of this new longitudinal artery. This longitudinal artery grows rapidly forward until it joins the corresponding descending artery opposite the midbrain. It is very clear in plate 6 that the longitudinal neural artery along the hindbrain is originally along the ventro-lateral border of the hindbrain, and thus that there

is one on each side. In plate 6 this vessel is labeled the basilar artery (*a.b.*), which is an illustration of the fact that the relations of the arteries of the adult may have too great an influence on the naming of the embryonic vessels. This vessel is not even a capillary which will become the basilar artery, because it is not in the mid-ventral line; it is rather a vessel which will become a part of a capillary plexus that will gradually reach the mid-ventral line, where the basilar artery will form. At the stage of plate 6 there are bilateral longitudinal arteries along the thalamus, the mid-brain, and the hindbrain, as can be proved by a direct ventral view of the specimen. The relations and the importance of this vessel would be emphasized by calling it a part of the primary longitudinal neural artery. On the other hand, the vessel shown in plate 7 from a pig embryo of an older stage is in the mid-ventral line and is thus the true basilar artery.

During the fourth day of incubation the longitudinal artery seen opposite the first, second, and third somites in plate 6 grows caudalward along the ventro-lateral surface of the spinal cord on either side, to the caudal end of the neural tube. These ventro-lateral arteries develop as a longitudinal anastomosis between all the segmental arteries of the spinal cord. At the stage of the fourth day of incubation it is clear that the vascular plexus along the entire surface of the neural tube is supplied with blood by bilateral ventro-lateral arteries which extend from the groove between the cerebrum and the thalamus to the caudal tip of the tube. These two longitudinal arteries are originally in the form of a plexus on either side of the subthalamus, as is still better shown in plate 7 for the pig, and are more definitely a single channel along the rest of the course.

This longitudinal neural artery receives its blood from the forerunner of the carotid arteries on either side and from the segmental arteries. It is easy to see that it is these important longitudinal arteries which will ultimately give rise to the circle of Willis, the basilar artery, and the anterior spinal artery.

The development of the anterior spinal artery has been worked out in the pig by Evans (1909 and 1912). In the chick the anterior spinal artery does not form until the fifth day of incubation. During the fourth day there are two ventro-lateral arteries along the spinal cord which are placed on either side of the notochord and are not connected except by an occasional capillary across the mid-ventral line; they make a sharp ventral boundary for the lateral plexus on the spinal cord. These two longitudinal arteries are just mesial to the point where the spinal arteries meet the spinal cord, as can be seen in Evans's figure 437 c in the "Manual of Human Embryology" (Keibel and Mall). They give rise to the characteristic anterior arteries which penetrate the spinal cord. During the fifth day of incubation these two longitudinal arteries become connected with each other across the mid-ventral line, which is the beginning of the formation of the anterior spinal artery. The stage of the fourth day of incubation for the chick in which there are bilateral longitudinal arteries along the ventro-lateral border of the entire neural tube from the point of origin of the carotid artery to the tip of the spinal cord is an important stage for understanding the blood-supply of the nervous system.

It must be made very clear indeed that the longitudinal artery seen along the hindbrain in plate 6 is a neural artery and is not the vertebral artery. This is a specimen of the third day of incubation and the artery shown in this specimen forms along the neural tube at a stage when the occipital arteries supply only the neural tube. On the fifth day of incubation, on the other hand, these same arteries also supply the corresponding myotomes with vessels, and there then forms a second longitudinal anastomosis on either side along the upper segmental arteries which is nearer the aorta than the neural vessel. These second longitudinal vessels become the vertebral arteries. These arteries form at the stage of the fifth day of incubation in the chick and are present in a pig measuring 15 mm., a very much older stage than the one shown in plate 7, which measured 6.5 mm. The vertebral arteries form as the heart is shifting farther caudalward; and indeed it is clear that the basilar and anterior spinal arteries together, as well as the vertebral arteries, provide for the arterial supply of the hindbrain when the shifting relations in the neck interfere with the direct arteries from the aorta. The fundamental relations of the neural arteries to the plexus on the surface of the neural tube has now become clear. This plexus is fed with arterial blood from bilateral longitudinal arteries which are along the ventro-lateral border of the plexus and eventually come to lie for the most part in the mid-ventral line. Over the surface of the subthalamus the vessels remain bilateral.

It is now necessary to consider how the neural plexus becomes related to the veins. In the study of the development of the veins of the brain as distinct from those of the spinal cord, it is of primary importance to study how the deep plexus of vessels becomes related to branches of the primary head-vein. This point I have worked out more in detail in the pig and shall therefore take up its consideration later. The fundamental points are, however, (1) that the branches of the primary head-vein opposite the forebrain and midbrain are transverse veins superficial to the deep plexus which constantly tap the deep plexus at their tips and grow toward the mid-dorsal line; (2) that the transverse veins of the hindbrain are profoundly influenced by the presence of the ganglia of the hindbrain and by the otic vesicle. The sensory ganglia become as completely surrounded by a capillary plexus as the neural tube itself, and each of these plexuses gives rise to a vein or group of veins. Moreover, the same is true for the spinal ganglia.

In this account of the origin of the neural vessels great stress has been laid on the development of the vessels of the hindbrain, on account of the peculiar relations of the primitive vessel of the hindbrain to the drainage of the forebrain. In the course of the development of the vessels of the hindbrain the direction of the circulation of the blood is ultimately exactly at right angles to its original course. This change takes place, (1) by the completion of the true head-vein, by which the pial vessel is relieved of a great volume of venous blood from the brain; (2) by the development of a new longitudinal arterial channel, by which it can receive a much greater arterial supply. By these changes the blood over the hindbrain soon runs from the ventral toward the dorsal border, at right angles to its original course from the cephalic to the caudal border.

In the transition from the stage in which the primitive channel of the hindbrain serves as the vein of the brain to the stage when the new lateral superficial vessel—the true primary head-vein—is complete, it is clear that the primitive transverse vein of the first interspace is cut out of the main line of drainage for the head. It does not form a part of the primary head and neck vein of the embryo. Thus the primary head-vein, from the standpoint of development, consists of three parts: an anterior division, which is the primary cerebral vein; a second portion, which is a true head-vein draining the entire brain, forebrain, midbrain, and hindbrain, as well as the visceral arches; and thirdly, the anterior cardinal vein. The transverse vein of the chick persists as a root of a characteristic vein of the hindbrain—namely, a vein which arches caudalward along the lateral surface of the medulla. This vein of the medulla will be followed farther in the pig. It was called the posterior cerebral vein by Mall. The position of the transverse vein of the chick embryo in the first interspace is also just opposite the cephalic end of the ganglia of the vagus nerve. As soon as the superficial vein—the primary head-vein—is formed, the vascular channel of the neck straightens out, and there is then no longer any way of distinguishing the exact place where the second segment of the primary head-vein joins the third segment or the anterior cardinal vein, for the two become a single, continuous channel. From now on, the place of transition can be indicated only in a general way by the root of that vein of the medulla which follows the roots of the vagus nerve along the medulla; and it is well known that veins are shifting landmarks. Stated in other words, the anterior cardinal vein extends along the entire zone of the occipital myotomes, and as the occipital muscles develop these myotomes become indistinct landmarks.

The first interspace is thus a transitory landmark, and in later stages and as soon as the superficial head-vein connects directly with the anterior cardinal vein and eliminates the transverse vein of the first interspace from the direct line of drainage for the blood of the brain, the distinction between the head-vein and the anterior cardinal vein becomes less obvious. The cephalic portion of the head-vein develops to drain the forebrain and midbrain; the middle portion develops to drain the brain and the gill-arches. The vein of the anterior part of a chick of the fourth day of incubation is therefore a composite structure, so far as development is concerned. However, at the fourth and fifth day of incubation there is a single long vein extending from the groove between the cerebrum and the thalamus down to the duct of Cuvier. This vein receives branches from all the various structures of the head. The neural branches come from the cerebrum and the eye from the thalamus, the midbrain, and the hindbrain. The branches from the hindbrain are especially modified by the ganglia of the hindbrain and the otic vesicle. On the ventral aspect this vein receives branches from the developing visceral arches and from the somatopleure opposite the heart. The entire vein may thus be called the embryonic head-vein, or the vena capitis prima.

As far as the relations of the vena capitis prima to the vessels of the adult are concerned, it has been shown by Mall and Streeter that only a very small portion of the primary head-vein persists within the skull cavity—namely, the segment just mesial to the Gasserian ganglion which becomes the cavernous sinus. The neural branches of the primary head-vein ultimately give rise to the other dural sinuses.

In regard to the relations of the anterior cardinal vein of the embryo to the internal jugular vein, it is interesting to note, in plate 6, that the entire anterior cardinal vein is opposite occipital myotomes; that is, it is entirely within the head. The caudal part of the anterior cardinal vein will become a vein of the neck when the duct of Cuvier shifts into the zone of the cervical myotomes. The cephalic end of the anterior cardinal vein of the embryo is opposite the upper zone of the medulla. The cardinal system of veins in general covers the entire zone of the myotomes, which includes a part of the head as well as the entire body of the embryo.

In closing this account of the origin of the primary head-vein, it is important to emphasize again the relation of the new vessel, the middle portion of the vena capitis prima, to the various structures related to the hindbrain—that is, to the otic capsule and to the ganglia of the hindbrain. The middle portion of the head-vein develops after these structures are formed and must conform to their position. It grows in as straight a line as possible, and passes mesial to the placode of the trigeminus, lateral to the acoustic complex, to the otic capsule, and to the ganglion of the glosso-pharyngeus. It is entirely a new vessel, and has no remnants whatever of the preliminary vascular channel of the hindbrain which arises and runs along the neural tube. As is seen in plate 6, there are two entirely distinct vessels in the head of a chick of the early part of the fourth day—the so-called vena capitis mesialis, a neural vessel, and the so-called vena capitis lateralis, a true head-vein.

After following this account of the origin of the primary head-vein of the chick, it will be of value to consider the long series of previous studies upon which it has been based. The observations which seem to me to lead to a clear understanding of this subject are those of Salzer, Mall, Grosser, Evans, Williams, and Streeter. The view first held in regard to the development of the veins of the head was that the external jugular vein was the primary vein of this region. This view, which was incorrect, was based on the work of Rathke. In 1887 Kastschenko described a remarkable relationship between the jugular vein and the cranial nerves in the chick. He stated that up to the end of the third day the cranial nerves were lateral to the jugular vein (primitive vessel of the hindbrain), and noted that this vein was not in the form of a plexus. At the end of the third day the facial and glosso-pharyngeal nerves became mesial to the vein, and on the sixth day the vagus became mesial. He thought that the nerves cut through the veins, as it were, without the latter losing their continuity.

In 1895 Salzer published an article on the development of the veins of the head in the guinea-pig, which forms the basis of the correct interpretation of this

difficult subject. He described the head-vein, in an embryo guinea-pig 2.5 mm. long, as a vessel running from the region of the optic cup, close to the neural tube (primitive vessel of the hind-brain), to the level of the first vertebra, where it turned lateralward and lay lateral to the aorta (anterior cardinal vein), ending in the duct of Cuvier. This vein (the primitive vessel of the hindbrain), was mesial to the cranial nerves, and Salzer called it the anterior cardinal vein. It was a transitory vein, for by the time the embryo was 2.8 mm. long he found a second vein, lateral to the nerves, from the region of the acoustico-facial complex forward. This second vein he called the vena capitis lateralis, and concluded that, not only in the guinea-pig but in vertebrates in general, the anterior cardinal vein (deep channel of the hindbrain) is the first vein to develop in the head, and that it is replaced by a vena capitis lateralis, which as the neck develops is continued into the neck as the internal jugular vein. This description of the veins of the early embryo by Salzer is nearly correct, and was a great step in advance, though more complete studies give a different interpretation and naming of the veins.

The next step was made in 1907, by Dr. Mall, who studied the cerebral sinuses in the human embryo and, on the basis of this work of Salzer, demonstrated that the first drainage canal for the head (primary head-vein including the anterior cardinal) gives rise to the cerebral sinuses and the internal jugular vein. This drainage canal (the vena capitis prima) he called the anterior cardinal vein, using the term in its generally accepted sense as applying to the entire head-vein and neck-vein of the embryo.

In the same year Grosser made it clear that the first vascular channel for the head (deep vessel of the hindbrain and the anterior cardinal) can be analyzed into two parts: a cephalic part which lies close to the neural tube, and a caudal part which has an entirely different position—namely, ventral to the myotomes and lateral to the aorta, in the same position as the posterior cardinal vein. He limited the term "anterior cardinal vein" to this caudal portion, and analyzed the cerebral portion into a primary vessel (the vena capitis medialis) and a secondary vein (the vena capitis lateralis).

At this point Evans gave his beautiful injections of early blood-vessels, published in 1909. He showed the form of the primitive vascular plexus of the brain and also how this plexus covered the surface of the forebrain, encircling the large optic vesicle with a chain of capillaries and spreading over the surface of the thalamus and midbrain. He described how this extensive plexus became a single slender channel along the wall of the hindbrain, leading down to the transverse vein and the duct of Cuvier; and also demonstrated the connections of the plexus of the forebrain and the single vessel of the hindbrain with the aorta.

Streeter has recently published a study dealing with the later stages of the vena capitis prima. It was from the branches of this vein that Dr. Mall had shown that the dural sinuses were derived. Streeter has worked out the development of the dural sinuses more in detail and has shown that the only part of the vena capitis prima to persist is the part mesial to the Gasserian ganglion which

becomes the cavernous sinus. The rest of the dural sinuses come from branches of the primary head-vein. The sinuses of the mid-dorsal line arise from the anastomoses of the veins of the two sides; the basal sinuses arise for the most part from veins which border the Gasserian ganglion and the otic capsule.

In 1916 Stracher published an article on the veins of the head of the chick, in which he deals with the fate of the vena capitis medialis and the origin of the vena capitis lateralis. In this work he uses the method of reconstruction in preference to the method of injection in a form in which it is easy to obtain abundant injected material, on the ground that with reconstructions the relations to the surrounding tissues can be better analyzed. Stracher's own work, however, suffers from the limitations of his method. His reconstructions show the larger trunks, which are not always the most important ones, and do not show certain tiny channels which are essential to an understanding of the relations of the vessels. He shows the stage at which the primitive vessel of the hindbrain (vena capitis medialis) and the primary head-vein are both present in the same specimen and equal in size. This had not been done previously, and is an important point. He also shows in part how the middle segment of the primary head-vein arises, but misses several points that are essential to an understanding of this vein. In his text-figure 2, from a chick of 30 somites, he shows a short branch from the anterior cardinal vein and a branch from the maxillary (ophthalmic) veins, and recognizes that these two branches become connected and form the vena capitis lateralis. He speaks of the branch from the inferior orbital vein (my maxillary vein) as arising from a swelling on the vena capitis medialis, not realizing that it is a new outlet, not for the blood of the vena capitis medialis but for the blood of the primitive cerebral vein, as is plainly shown in plate 6. In discussing the origin of the vena capitis lateralis from the lower border of the Gasserian ganglion to the anterior cardinal vein he says (page 55):

> Kastschenko gibt keine Abbildung, die ihre Entstehung zeigen würde, seine Tafel stellt sie da, nachdem ihre Ausbildung vollendet ist. Nach seiner Schilderung "durchschneiden" die Nerven die Vene. Demgegenüber ist zu betonen, dass der eben geschilderte Teil der Vena capitis lateralis—es folgt später noch die Ausbildung weiterer caudal and cranial davon gelegener Strecken—frei im Gewebe, ziemlich entfernt von der Vena capitis medialis entsteht.

Thus he realized a part of the method of origin of the vena capitis lateralis, but missed entirely its relation to the capillaries of the visceral arches. In regard to the relations of the portion of the primary head-vein in the region of the Gasserian ganglion, Stracher's models are better than his interpretations. The essential facts are that the vena capitis medialis is a vessel on the hindbrain, the vena capitis lateralis is a more superficial vein which lies ventral to the hindbrain; both are present in the same specimen at a given stage; both are mesial to the Gasserian ganglion, one as a part of the system of vessels of the pia mater and the other as a part of the primary head-vein.

Stracher shows both the vena capitis medialis and the vena capitis lateralis in their correct position mesial to the Gasserian ganglion, and then concludes

that the medial vein of this area becomes transformed to make the lateral vein. His own figures do not warrant this conclusion, which was formed through not following the fate of the primitive vessel of the hindbrain. Thus his diagram (page 68), which should show the primary head-vein coming, embryologically, from three segments—namely, from (in his nomenclature) the vena cerebralis anterior, the vena capitis lateralis, and the vena cardinalis anterior, shows it coming from five: (1) the vena cerebralis anterior; (2) a short stretch of the vena capitis lateralis; (3) the vena capitis medialis; (4) the vena capitis lateralis again; (5) the vena cardinalis anterior. He observed the beginning of the breaking of the vena capitis medialis into a plexus, and then missed the plexus as it became finer, so that he lost the very important point of the fate of the primitive channel of the hindbrain. These points are covered in his summary (page 67):

"Sodann entwickelt sich eine neue Venenbahn (Vena capitis lateralis), die parallel zur medialen Kopfvene, aber lateral vom Nervus acustico-facialis, glosso-pharyngeus und dem Hörbläschen verläuft. Sie verbindet das Stück der Vena capitis medialis, das sich medial von der Trigeminusanlage findet, mit der vorderen Kardinalvene. Zur selben Zeit weiten sich Gefässe des Venennetzes am Hinterhirn zu einer Bahn aus, die bedekt von der Trigeminusanlage beginnt, an der Seite des Hinterhirns dorsal von Hörbläschen im Bogen verläuft und in der Gegend des Nervus glosso-pharyngeus wieder zur medialen Kopfvene zurückkehrt (Vena capitis dorsalis). Sie tritt mit Beginn der Obliteration der Vena capitis medialis auf und verschwindet wieder, sobald die Vena capitis lateralis vollständig ausgebildet ist. Die Vena capitis medialis verödet zuerst im Bereich des Hörbläschen, dann caudal davon in der Gegend des Nervus glosso-pharyngeus. Weiterhin entwickelt sich dadurch um den ersten Ast des Nervus trigeminus ein Venenring, dass lateral vom Nerven eine Vene entsteht, die rostral vom Nerven aus dem Stamm austritt und sich caudal wieder mit ihm vereinigt. Der mediale Schenkel dieses Ringes verschwindet alsbald. In ähnlicher Weise entwickelt sich auch um den Nervus vagus ein Ring mit Beihilfe der dorsal einmündenden Zweige. Auch hier verödet die alte medial vom Nerven gelegene Bahn. Damit is die Vena capitis lateralis vollständig ausgebildet, und die Kopfvene ändert ihren Verlauf, was ihre Lage zu den Nerven anlangt, nicht mehr, da die Nervi accessorius and hypoglossus beim Huhn auch im ausgebildeten Zustande lateral von Vena jugularis interna ziehen."

It is, I think, clear that the primary blood-vessels which arise in the head are neural vessels. These neural vessels form a continuous plexus of capillaries which closely invests the brain. Along the hindbrain angioblasts probably appear first, but here they form a single, characteristic long channel which serves temporarily as a vein and does not take the form of a plexus characteristic of the neural vessels until relatively late. This single, large, primitive vessel does not extend the full length of the rhombencephalon, however, but at the zone of the cephalic roots of the vagus nerve, or, in other words, opposite the first occipital myotome, becomes a plexus on the side of the medulla which gradually extends the full length of the cord and connects with every intersegmental artery and vein. The neural system of vessels becomes connected with the venous end of the heart by means of the two cardinal veins. These connections are very characteristic; the most cephalic, which is either in front of the first occipital myotome (as in the pig) or between the first two occipital myotomes (as in the chick), is always the largest and drains the entire brain. All the other connections are

small intersegmental veins. Thus it may be said that two organs determine the early blood-vessels, the neural tube, and the nephrotome. Soon a third set of organs (the visceral clefts) develop and give rise to capillaries, which connect on the one hand with the anterior veins of the brain and on the other with the cardinal veins; and in this manner the head-vein of the embryo is completed.

The method of nomenclature of the primitive vessels of the head is certainly open to discussion. The primitive vessel of the hind-brain, of which I have shown the origin, the relations, and the fate, is the vessel seen by Kastschenko in 1887 and more clearly by Salzer in 1895, and recognized by all who have since worked on this subject as being the first long vein in the head region and as lying along the wall of the hindbrain. Grosser gave it the name of "vena capitis medialis," a name which has been universally accepted.

It may be argued that it is a mistake to attempt to change a name of this type which has been generally adopted; but on the other hand a name which would emphasize the essential point in regard to this vessel—namely, that it is a neural vessel and that it develops into neural vessels—rather than the accessory fact that it serves temporarily as a vein for the head, would, I am convinced, clear up much of the confusion in regard to the primitive veins of the head. It does serve for two days in the chick as a vein for the forebrain and the midbrain, but at the same time it is the entire capillary bed of the hindbrain and ceases to be a single long channel as soon as the cerebral blood is shunted through another channel. It then develops, as have the rest of the neural vessels, into an extensive capillary plexus in the position of the pia mater. I therefore wish to avoid the use of the term *vein* in connection with it and to reserve the term *vein* for the vena capitis lateralis, for which I shall use the term *vena capitis prima*, because this is the first vascular channel of the head which is purely a vein and because it is the first vessel which drains the head and not the brain alone. I have therefore called the vena capitis medialis the primitive vessel of the hindbrain. The term *vessel* is more indefinite than the term *vein*, but for that very reason it applies better to a channel which serves both as a vein and as a capillary at the same time, and ultimately becomes a capillary plexus, out of which both arteries and veins will arise. I propose to call the long vein of plate 6, extending from the region of the thalamus to the duct of Cuvier, the primary head-vein. This primary head-vein develops in three segments—a cephalic segment which is the primitive cerebral vein, a middle segment opposite the hindbrain, and a caudal segment which is the vena cardinalis anterior. At the stage of plate 6 this vein is entirely within the head, because the duct of Cuvier is still opposite occipital myotomes. As soon as the duct of Cuvier shifts into the neck region this vein will become the primary head and neck vein. The terms *vena capitis medialis* and *lateralis* have the sanction of usage; but it seems to me that the terms *primitive vessel of the hindbrain* and *primary head-vein* better express the function of these vessels.

## DEVELOPMENT OF THE SPINAL ARTERIES.

It will now be necessary to go back and follow the development of the spinal arteries. This can be done in a series of injections of the stages from 15 or 16 somites upward, or the entire process can be followed in any one chick up to the stage of about 30 somites. The process is easier to illustrate after the embryo has rotated so that the lateral instead of the dorsal surface is presented. The entire process can thus be readily followed in plate 3, figure 1, from a chick of 25 somites, with six sections from two different series of chicks of 25 and 30 somites.

The general stage of development of the vessels of the head of the embryo at the stage of 25 somites can be seen in Evans's figure 6 (Anat. Record, 1909, III, p. 505); or can be estimated from my plate 6, the stage of 25 somites being just before the superficial capillaries which make the middle segment of the primary head-vein begin. The deep vessel of the hindbrain is still the vein for the brain, and is shown in its relation to the capillary plexus on the lateral surface of the spinal cord in plate 3, figure 1.

The general development of the area vasculosa at this stage is also of interest in following the vessels in sections. The roots of the omphalo-mesenteric arteries at the stage of 25 somites are opposite the twentieth and twenty-first somites. As was indicated above, in the earlier stages the entire lateral border of the aorta opposite the somites was originally connected by direct lateral (that is, ventro-lateral) branches with an arterial plexus of the area vasculosa. In this plexus, on either side of the embryo, the omphalo-mesenteric veins gradually extend caudalward from the region of the sinus venosus and thus are formed two veins, or a plexus of veins, with direct short connections with the aorta. This process explains the large veins of the splanchnopleure shown in figure 3, plate 2, and figures 2 and 3, plate 3.

In the chick the spinal arteries do not arise as direct dorsal arteries from the aorta to the cord, but the direct dorsal arteries make a primary arch to the dorsal border of the nephrotome, where they give rise to the cardinal veins. The spinal arteries then arise from these arches instead of from the wall of the aorta itself.

In following the development of the spinal arteries I shall begin with the more caudal segments in plate 3, figure 1, because they show the earlier stages. As has been described in connection with the origin of the cardinal veins, the first dorsal branches of the aorta are direct dorsal diverticula of the wall of the aorta into the interspaces, as is shown best in text-figure 3 for the stage of 12 somites. Figure 2 on plate 4 and figure 4 on plate 2 are both from the lower segments of a chick of 30 somites. They are both taken below the origin of the omphalo-mesenteric arteries in the zone where the arteries of the posterior limb-buds are forming. The cardinal veins are also developing in this area. Figure 2 on plate 4 passes through the twenty-fifth interspace, and plate 2, figure 4, is still lower down and passes through the twenty-seventh interspace. In plate 4, figure 2, it can be seen that even in later stages the dorsal branches start as direct diverticula of the aorta. These diverticula soon arch lateralward and, as can be seen in plate 2, figure 4, dilate slightly just dorsal to the nephrotome. These dilated portions of the arches

become connected with similar dilatations in the other interspaces and make the cardinal veins. These observations indicate that the cardinal veins begin with dilatations of the dorsal branches of the aorta—that is, that they start as an outgrowth from the wall of the aorta in the different interspaces and that these intersegmental vessels become connected along the lateral line.

Very soon the vascular arches which give rise to the cardinal veins give off sprouts which extend toward the spinal cord, as is shown from the tenth to the seventeenth interspaces in plate 3, figure 1. The position of these sprouts is shown in section on plate 2, figure 3. This section passes through the twenty-first interspace of a chick of 30 somites. These neural sprouts soon reach the spinal cord, as is shown in section on plate 3, figure 4, which is to be compared with the seventh interspace of plate 3, figure 1. The section shown in plate 3, figure 4, is from a chick of 25 somites—from another series than that of all the other sections on the plates. It is from a series of nearly the same stage as that of plate 3, figure 1; it passes through the seventeenth interspace. It was selected because it shows so well the double dorsal arch to the posterior cardinal vein, the primary direct one and the secondary neural one. One has only to imagine the primary direct arch disappearing to obtain the well-known pattern of the spinal arteries shown in section on plate 3, figure 2, and in the upper interspaces of plate 3, figure 1.

From this study it is, I think, clear that the spinal arteries of the chick arise from dorsal intersegmental vessels which give rise to the cardinal veins, and not directly from the aorta. In plate 3, figure 1, it is very evident that the capillary plexus which forms along the lateral surface of the spinal cord is a direct continuation of the primitive vessel of the hindbrain. The original simple chain of capillaries on the lateral surface of the cord, such as is shown in plate 3, figure 1, from a chick of 25 somites, very soon becomes a plexus on the neural tube, as indicated opposite the second somite in plate 6. By the fourth day of incubation this plexus covers the entire lateral surface of the spinal cord. The relation of this plexus to the spinal arteries on the one hand and to the spinal veins on the other is very regular and characteristic. Every spinal artery, on approaching the cord, bifurcates into a short ventral branch and a longer lateral branch (plate 3, fig. 2). The ventral branch leads to a longitudinal neural artery which at the stage of the fourth day lies on the ventral surface of the cord just lateral to the notochord. In other words, there are symmetrical ventral longitudinal arteries. These arteries form a ventral border for the plexus along the lateral surface of the cord. The lateral arteries run in the plexus on the surface of the cord; they lie just cephalic to each spinal ganglion and extend nearly to the dorsal border of the cord. The veins which accompany these transverse arteries, in contrast to the arteries, are lifted off from the surface of the cord, as it were. They also correspond to the cephalic border of each ganglion, and they are more superficial in every case than the corresponding artery. I am emphasizing the fact that the arteries lie in the plexus on the surface of the cord and that the veins are more superficial, because the same is true for the primary arteries and veins of the brain, as can be seen very clearly in plate 7.

The series of sections of injected chicks shown on plates 2, 3, and 4 allow an interesting comparison of the primitive branches of the aorta. It is clear that the branches of the aorta can be described best according to what organs they supply rather than by regarding their exact point of origin in the wall of the aorta. The primary branches extend to the splanchnopleure and are primitively directly lateral branches, as can be seen on the left side of text-figure 3. That they come to be ventro-lateral and then ventral branches is well known and is shown in plate 2, figure 4, in which it is clear that there are three sets of arteries on the right side of the section. The first is a dorsal branch to the cardinal vein; the second is a lateral branch to the somatopleure, and the third is a ventral branch to the splanchnopleure. The branches that extend to the cardinal veins need careful attention. There are in the first place the original dorsal arches that give rise to the cardinal veins, such as are shown in figure 4 of plate 2 and figure 4 of plate 3. These branches are strictly intersegmental; moreover, the intersegmental branches are the first arteries related to the cardinal veins because they are the only ones present at the stage of 12 somites. In later stages (for example, at the stage of 25 or 30 somites) there develop a few direct dorso-lateral arteries to the cardinal veins, and these arteries may lie opposite the somites instead of between them. Such an artery, for example, is shown on the left side of figure 3 of plate 3 This section is the next one below that of figure 2 of plate 3 in the series and indeed the edge of this artery is shown in the latter section. Similar direct dorso-lateral arteries to the cardinal veins are shown on both sides of figure 4 of plate 3. This latter figure demonstrates that these dorso-lateral arteries are new vessels and not remnants of the original dorsal arches. On the left side of figure 4 of plate 3 blood reaches the cardinal vein in three ways: from the aorta along the surface of the cord, from the aorta along the primary dorsal arch, and from the aorta through a dorso-lateral artery. It must also be brought out that these dorso-lateral arteries to the cardinal vein are not the same as the direct lateral arteries to the tubules of the pronephros and the metanephros, which develop later and are quite differently placed, as can be seen in text-figure 8 from a chick of 35 somites. These dorso-lateral arteries to the cardinal veins are of importance in connection with the extension of the cardinal veins caudalward and are very important in comparing the chick with a form like the pig, where the dorso-lateral branches are more numerous.

It may be well to enumerate here the different types of branches of the aorta which may be found in the embryo from the standpoint of the structures they supply: first, there are the arteries to the splanchnopleure; second, mesial branches which connect the two aortæ; third, lateral arteries to the somatopleure leading to the umbilical veins; fourth, dorso-lateral arteries to the cardinal veins; fifth, lateral arteries to the limb-buds; and sixth, lateral arteries to the nephritic tubules.

## THE VASCULAR SYSTEM IN YOUNG PIG EMBRYOS.

In the study of the vascular system in a mammal it is not as easy to obtain young stages for injections, as in the case of the chick. The material, however, offers valuable opportunities for comparison with human embryos, and to obtain injections in much earlier stages than have ever been injected in human specimens. I shall follow the development of the vessels in the pig by the aid of six figures of injected embryos, and shall describe the specimens and follow the development of the vessels under six headings: First, the form of the heart; second, the ventral branches of the aorta, including the allantoic arteries and the subintestinal artery; third, the umbilical veins and the vessels of the thoracic body-wall; fourth, the vascular system of the nervous system and the formation of the primary head-vein; fifth, the cardinal veins; sixth, the vessels of the pronephros and the mesonephros.

### THE FORM OF THE HEART.

The youngest pig which I have injected is shown on plate 4, figure 3. This is from a specimen which measures 4 mm. in oil and which has 14 somites. It corresponds in development with a human embryo, No. 470 of the Carnegie collection, which measures 3.3 mm. and is in the fourth week of development. In this embryo pig an injection was made into the aorta opposite the origin of the omphalo-mesenteric arteries. The point of injection was obscured by extravasation, so that it is not shown in the drawing. The stage of development of the specimen can be judged by the form of the brain, the otic vesicle, and the form of the heart.

The extensive venous plexus covering the anterior or cephalic wall of the yolk-sac converges on either side into large right and left omphalo-mesenteric veins, which meet in a conjoined tube, the sinus venosus. The sinusoids of the liver have not yet begun to form, so that the sinus venosus stands out clearly. The sinus has a marked diverticulum, which Tandler called the horn. The dorsal wall of the sinus shows a series of sprouts, representing the duct of Cuvier, which is probably developed at this stage, as indicated by the posterior cardinal vein, but is incompletely injected. The most caudal of the sprouts form a small plexus representing the umbilical vein in the somatopleure.

Above the sinus venosus is a well-marked groove between the sinus and the atrium. The atrio-ventricular canal, on the other hand, is only just indicated. The form of the heart corresponds closely with the description by Tandler (Manual of Human Embryology, Keibel and Mall, page 536), which is based on the studies of Born, in which he says that the heart becomes a horizontal loop, the two limbs of which are separated by an almost horizontal bulbo-ventricular cleft into two parts, a ventricular limb and a bulbar limb. In my specimen the bulbar limb consists of three parts: first, the bulbus cordis; second, a short constricted portion of the tube, the fretum Halleri; third, the large truncus arteriosis, which gives off the two aortæ. In the use of the term *fretum Halleri* I am following the usage of His (Anatomie menschlichen Embryonen, pages 131 and 140). He describes this portion of the tube as the portion which ultimately gives rise to the semilunar valves.

In connection with the development of the heart, figure 1 of plate 5 and figure 1 of plate 1 are very interesting. The specimen from which the former was taken was one of a litter of five, all of which were injected. It measures 6 mm. in oil, that is, after fixation and dehydration, and has 20 somites. The specimen on plate 1, figure 1, was one of a litter of six embryos, all of which were injected. It measured 7 mm. when fresh and is 6.2 mm. long in oil. It has 23 somites. It should be noted that these embryos do not have a caudal flexure, so that these measurements must not be confused with the same measurements of older specimens after the flexure has formed. The number of somites gives more valuable data in regard to the stage of development in these stages than do measurements.

If comparisons are made with human embryos at the stage of 23 somites it will be noted that at this stage the human embryo has two very marked flexures, shown, for example, in the R. Meyer embryo No. 300, represented in Felix's figure 531, in the "Manual of Human Embryology" (Keibel and Mall), and hence it is very much shorter.

In figure 1 of plate 5 the changes in the heart from the stage shown in plate 4, figure 3, are readily followed. The direction of the ventricular arch has changed from the horizontal to an oblique position. The atrio-ventricular canal has become the characteristic long, slender channel, and there is a marked constriction between the ventricle and the large bulbus cordis. The fretum Halleri is now a long, slender tube, and both the bulbus cordis and the truncus arteriosus are shown in maximum distension.

In plate 1, figure 1, the form of the sinus venosus is not clear, as it is concealed by the injection of the sinusoids of the liver. In all of the six specimens of this litter the sinusoids of the liver are farther developed on the left side than on the right. In all of the other specimens, however, and on the right side of this specimen, there is a marked constriction between the liver and the sinus venosus just below the upper large opening of the umbilical vein. At this stage the umbilical vein connects with the liver and with the sinus venosus by large openings, and with the duct of Cuvier by an extensive capillary plexus in the somatopleure. There is a constriction between the sinus venosus and the atrium, and a well-marked atrio-ventricular canal. The bulbo-ventricular cleft gives the effect of an hour-glass constriction of the heart. This is true of all the specimens of the litter, but in one the contraction of the bulbar portion is particularly marked. The differences in the form of the heart in figure 1, plate 5, and figure 1, plate 1, are partly due to the fact that the hearts in these specimens were fixed while beating and were caught at different phases of the beat. For example, in plate 5, figure 1, and bulbus cordis and the truncus arteriosus show a maximum distension, while in plate 1, figure 1, the bulbus cordis and the truncus arteriosus are contracted and there is a general distension of the cephalic aorta. On the other hand, in plate 1, figure 1, is shown the beginning of a torsion of the ventricular loop, by means of which the beginning of the fretum Halleri will come to be opposite the ventricular end of the atrio-ventricular canal.

This torsion is more clearly seen on figure 2 of plate 5 and figure 1 of plate 4. These two specimens are from the same litter. They measure 7.1 mm. in oil, and have 27 somites. Figure 1 of plate 4 is given because of an extravasation in the vessels of the head in the specimen of figure 2 of plate 5. In this latter figure the sinusoids of the liver have markedly developed. The sinusoids of the left side anastomose across the ventral line with those of the right side. The opening of the left umbilical vein into the liver is directly mesial to the umbilical vein itself and is hidden by it, while the opening into the duct of Cuvier is plainly visible. There is also a plexus from the umbilical vein in the somatopleure connecting it with the posterior cardinal vein and with the duct of Cuvier; but this is omitted in the drawing.

There is a well-marked constriction between the sinus venosus and the atrium. The change in the heart is due to the twisting of the obliquely placed ventricular arch, whereby the point which marks the beginning of the fretum Halleri comes to lie exactly opposite the opening of the atrio-ventricular canal into the ventricle. The bulbus cordis lies far to the right and its connection with the fretum Halleri is hidden by the ventricle, while the opening of the auricular canal is far to the left. These relations as seen from the other side are shown in plate 4, figure 1. From these two figures it is obvious that a still further twisting of the heart must take place before the arterial orifice comes to lie directly anterior.

## VENTRAL BRANCHES OF THE AORTA, INCLUDING THE ALLANTOIC ARTERIES AND THE SUBINTESTINAL ARTERY.

One of the most interesting subjects in connection with these injections has been the study of the ventral branches of the aorta, or the branches to the yolk-sac, the gut, and its derivatives.

The study of the early vessels of the embryo emphasizes the fact that the vessels should be considered in relation to the organs which they supply. The fundamental relations of the ventral branches of the aorta to the yolk-sac and to the allantois are shown in two total preparations of injected pig embryos (plate 5, fig. 1, and plate 1, fig. 1) and in two sections (text-figs. 5 and 6). Plate 5, figure 1, is from a specimen of approximately the same stage as in Evans's figure 394 in the "Manual of Human Embryology," which shows the state of development of vessels of the brain at this stage.

The position of the embryo should be carefully noted. The caudal half of the specimen is seen from the direct ventral aspect, while the cephalic half is from a direct lateral view. The place of rotation is around the ninth somite.

Extending from the level of the eleventh somite to the caudal end of the embryo there is a series of tiny ventral arteries from the two aortæ. These are of uniform size and are placed at regular intervals, approximately one opposite an interspace and one opposite a somite. In this particular embryo only a few of these ventral branches are injected; but other specimens show that the entire length of both aortæ gives rise to branches like those shown opposite the twelfth, thirteenth, and fourteenth somites. From the region of the eleventh to the four-

teenth or fifteenth somite these tiny branches from the two aortæ unite in a plexus of large arteries on either side of the stalk of the yolk-sac, which join and give rise to the omphalo-mesenteric arteries on the yolk-sac. The large arteries are seen only on one side in plate 5, figure 1, and plate 1, figure 1, but are shown on both sides in figure 2 of plate 5. From the fourteenth somite caudalward the ventral branches of the aorta are uninjected in this specimen (plate 5, fig. 1), but show in other specimens leading to a single artery which arises in the caudal end of the embryo. Opposite the caudal end of the embryo the ventral branches of the aortæ form a sheet of capillaries on either side of the alimentary canal, which deserves careful consideration. These two sheets of capillaries form a plexus which completely surrounds the entire caudal end of the gut cephalic to the allantois, the stalk of the allantois, and the blind end of the gut, caudal to the allantois. This capillary plexus gives rise to two arteries, the paired allantoic arteries and the single subintestinal artery. Thus, we have here examples of arteries in the embryo which arise in a capillary plexus and end in a capillary plexus. The primitive allantoic arteries arise in a plexus around the stalk of the allantois and pass to the capillaries of the body of the allantois; the subintestinal artery arises in a capillary plexus around the gut and runs to the capillaries of the yolk-sac.

The allantoic arteries, as seen in plate 1, figure 1, extend into a plexus on the ventral or cephalic surface of the allantois; this plexus arches around the dome of the allantois, though not completely shown in the drawing, and reaches the veins on the caudal surface. The two allantoic veins join the umbilical veins at the point where the stalk of the allantois is fused with the body-wall. A section through the allantoic arteries from an injected embryo of the same litter as the specimen of plate 1, figure 1, is shown in text-figure 6, and shows the allantoic arteries following the wall of the gut into the allantois. In the series from which text-figure 6 is taken there are a few tiny capillaries extending dorsalward from the allantoic arteries just at the point where these arteries pass ventral to the cœlom. These capillaries grow lateral to the cœlom, and when the posterior limb-buds begin they will anastomose with the iliac arteries. These capillaries will become the umbilical arteries in the somatopleure.

These observations on the pig agree with the findings of Hochstetter in the rabbit (1890) and show that in these forms the primary allantoic arteries are vitelline vessels, while the central ends of the umbilical arteries are vessels of the somatopleure, which appear later and anastomose with the primitive allantoic arteries.

In the study of the R. Meyer human embryo 300, Felix (1910) gives an exceedingly interesting reconstruction of the vascular system of a human embryo which is of the same stage as my figure 1 of plate 1. This reconstruction (fig. 7, Morph. Jahrb, 1910, XLI, p. 590) shows that the primitive artery of the fetal membranes at the caudal end of the embryo arises in a capillary plexus around the gut, just as is shown in my figure 1 of plate 5 and figure 1 of plate 1. The position of this plexus in the wall of the gut is shown in section in Felix's figure 9, which is to be compared with my text-figure 6. The same relations are shown for the chick in Duval's Atlas, plate XXIII, figure 372. In the human embryo this artery

has been traced back as a vitelline vessel to the stage of 5 somites by Felix (1910), and to the stage of 8 somites by Dandy (1910). This artery in the wall of the gut, which is the primitive allantoic artery in the pig, has been called the umbilical artery in the human embryo on account of the insignificance of the allantois and the earlier vascularization of the chorion. The relations of these two vessels in connection with the human embryo were summed up by Evans (1912, page 595) in the phrase that the umbilical artery is merely a modified vitelline vessel. The entire question of the relation of the arteries for the fetal membranes at the caudal end of the embryo has centered around the position of the central end of the arteries with reference to the cœlom, as can be seen in text-figure 6; that is to say, whether the artery is mesial or lateral to the cœlom. In general, both in birds and in mammals there is a primitive artery mesial to the cœlom; that is to say, a splanchnic vessel, and a secondary vessel, the umbilical artery, lateral to the cœlom running in the somatopleure. Thus the vessels develop in the same manner in the different forms, for there is a primitive splanchnic artery followed later by an artery in the somatopleure, but there are variations in the relative importance of the allantois itself.

Besides the two arteries of the allantois, the two sheets of capillaries of the wall of the caudal end of the gut give rise to another artery. Extending forward from the stalk of the allantois, as seen in plate 5, figure 1, the two plexuses meet in a capillary plexus ventral to the caudal root of the yolk-sac. This plexus is continued as a single, ventral, subintestinal artery which joins the omphalo-mesenteric plexus opposite the fourteenth or fifteenth somite. The point where the subintestinal artery joins the omphalo-mesenteric plexus is the well-known intestinal landmark where the stalk of the yolk-sac joins the gut. A figure which gives a very clear understanding of these relations is Tandler's figure 1 in the Anatomische Hefte, 1904, $1^{23}$, page 192.

This subintestinal artery in the pig is the more interesting in view of the corresponding subintestinal vein in the chick, discovered by Hochstetter in 1888 and accurately described by him. He described its relations not only to the omphalo-mesenteric veins, but also to the intestinal and the allantoic vessels, and noted that it disappeared and that the left vein was larger than the right. A complete understanding of the development of this vein in the chick can be gained from the figures of Popoff (1894). As was mentioned in connection with the chick, during the early hours of the third day of incubation the entire capillary plexus of the area vasculosa caudal to the omphalo-mesenteric arteries must be regarded as an arterial capillary plexus down to the marginal vein, as shown in Popoff's plate v. During the last hours of the third day, as seen in Popoff's plate vi, branches of the omphalo-mesenteric vein gradually extend caudalward on either side of the embryo in the wall of the yolk-sac, and arch around the posterior end of the embryo; the left vessel starts ahead of the right and is always larger than the right. As these veins gradually extend backward into the territory of the pre-existing arterial plexus, forming more and more new connections with the plexus, they change the direction of the current of the blood in the

plexus (which has been away from the heart) to a direction towards the heart. The vein on the left side quickly extends to the marginal vein, making the single posterior vein of the yolk-sac of Popoff, which lies a little to the left of the mid-line, as shown in Popoff's plate VIII. The two lateral veins form an arch around the posterior end of the embryo; this arch is just cephalic to the point where the stalk of the allantois will develop.

On the third day of incubation there is a very extensive capillary plexus on either side of the posterior end of the gut, and beginning at the very caudal tip of the gut on either side are symmetrical ventral veins, which unite in a loop just cephalic to the base of the allantois and then run forward, at first as two veins and then as a single ventral vein in the ventral wall of the yolk-sac. The subintestinal vein is thus the primitive vein for the entire posterior end of the gut, for the caudal tip of the gut, the allantois, and the entire rectum and intestine up to the margin of the yolk-sac. Caudal to the allantois these vitelline veins receive the most caudal branches of the posterior limb-bud. This relation has been described by Evans (Anat. Record, 1909, iii). On the third day the umbilical artery develops around the somatopleure in connection with the posterior limb-bud and anastomoses with the primitive allantoic capillary plexus in the wall of the allantoic stalk. By the beginning of the fourth day the vessels in the stalk of the allantois show an exceedingly interesting relation. On either side there is one large artery coming from the aorta and now running in the somatopleure instead of in the splanchnopleure; but this artery is fed also from a capillary plexus in the wall of the splanchnopleure, which completely surrounds the stalk of the allantois and the caudal tip of the gut, and by a few capillaries of the somatopleure from the tail of the embryo, which capillaries, however, tend to drain more and more into the posterior cardinal veins.

These relations are clear in the light of the development of these vessels. There is at first a plexus of capillaries arising from the aorta and running in the stalk of the allantois, in which arise the primitive allantoic arteries; and secondarily, a capillary plexus in the somatopleure of the caudal end of the embryo, in which an umbilical artery develops. The umbilical artery joins the original allantoic artery in the fused area of allantois, somatopleure, and amnion (see text-fig. 6), and then the primitive allantoic arteries from the aorta become reduced again to a capillary plexus. Thus the allantois has a double arterial supply and a double venous drainage, the former in the wall of the gut and the latter in the somatopleure. The primitive allantoic arteries arise in a plexus of the splanchnopleure, and the corresponding venous return is through the subintestinal vein; the subintestinal vein anastomoses with the allantoic veins, but the direct continuation of the allantoic veins is into the umbilical veins, which develop in the somatopleure. Finally the umbilical arteries develop in the somatopleure, connect with the allantoic arteries, and soon bring most of the blood to the allantois.

The fate of the subintestinal vein in the chick is very interesting. If an injected chick of the fourth and fifth days be dissected so as to expose the caudal end of the gut and the straight posterior segment of the gut which leads up to the open bell of the yolk-sac, it will be seen that the entire wall of the gut is surrounded by a capillary plexus. At the caudal end of the gut and just cephalic to the stalk of the allantois the ventral vein has entirely disappeared in this capillary plexus, while farther forward it is still clear in the ventral wall of the gut, though freely connected with the plexus. It is clear also that this posterior segment of the gut is receiving new arterial and venous connections which grow in along the dorsal border at the cephalic end of the segment. The new artery is a branch of the omphalo-mesenteric artery given off just at the root of the yolk-sac; it extends caudalward along the dorsal wall of the gut and anastomoses with the aortic branches which are the forerunners of the inferior mesenteric arteries. The new veins are branches of the omphalo-mesenteric veins within the mesentery, the forerunners of the portal system. The entire subintestinal vein gradually disappears as a single channel by developing into the plexus of the wall of the gut. In this plexus it is clear that the direction of the flow of the blood in the wall of the gut is from the ventral toward the dorsal border, at right angles to the direction of the stream in the subintestinal vein.

It may seem curious that the pig should have a subintestinal artery in place of the well-established subintestinal vein of the chick. As has been shown, the subintestinal vein in the chick develops as a part of the process by which the primitive circulation of the yolk-sac, with arteries and veins as far apart as possible, becomes changed so that every zone of the area vasculosa is invaded by veins. The pig of plate 5, figure 1, represents the more primitive condition for comparison with Popoff's plate IV, in which the caudal part of the yolk-sac is still arterial.

The subintestinal artery of the pig can be seen in section in text-figure 5 from a pig of the same litter as the one shown in figure 1, plate 1; it receives numerous ventral arteries from the aorta, as does the corresponding vein in the chick; but it joins the omphalo-mesenteric arteries at the point of loop of the mesenteric arteries instead of the veins. This same artery is still present in the pig measuring 9 or 10 mm. after the caudal flexure has formed, at which stage it is breaking up into the capillary plexus within the wall of the gut. By the time the pig is 15 to 17 mm. long there is a new longitudinal artery in the dorsal wall of the gut, extending from the superior mesenteric artery caudalward and anastomosing with all the ventral aortic branches which represent the interior mesenteric artery. At the same time the accompanying venous plexus from the omphalo-mesenteric vein extends along the dorsal border of the gut. As this new blood-supply for the lower half of the intestine develops, the ventral vein of the earlier stages of the chick, or the ventral artery of the pig, becomes reduced to a part of the capillary plexus in the wall of the gut. It is interesting to note that in a pig of 9.5 mm. the ventral artery of the gut is also accompanied by a plexus of ventral veins, which correspond to the single ventral vein in the chick. Thus the difference in the two forms becomes readily understandable, for the invasion of that

part of the gut by the veins is merely relatively later in the pig, and the veins are thus much more transitory.

Branches of the omphalo-mesenteric veins growing down the mesentery begin early in the pig. These are shown in plate 4, figure 3. They are not seen in plate 1, figure 1, because uninjected in the specimen. In other specimens from the same litter there is a vein in the mesentery underneath the umbilical vein, as seen from the side, and joining the main omphalo-mesenteric vein at the lower margin of the liver. These branches are shown in plate 5, figure 2. The veins in the root of the mesentery anastomose with the mesial cardinal (subcardinal) vein as soon as it develops. This anastomosis was described by Hochstetter.

The ventral subintestinal artery here described was discovered by Ravn in 1894 in the rat and mouse. The vessel was also described by Evans in the pig (Manual of Human Embryology, Keibel and Mall, foot-note 56 on page 656). Ravn's description can be readily followed in my plate 5, figure 1, as he described the main omphalo-mesenteric artery arising in the caudal end of the embryo. Both Ravn and Evans describe this subintestinal artery as arising from the umbilical artery. My specimens, however, are from still earlier stages, and prove that this vessel arises, as does all the rest of the omphalo-mesenteric system, in the wall of the yolk-sac or gut; that it is a true vitelline vessel. Its anastomosis with the umbilical arteries in the somatopleure occurs later. Thus the subintestinal artery in the pig and the subintestinal vein in the chick are vitelline vessels. They disappear as single channels and help in the formation of the primitive plexus in the wall of the gut in connection with the changes by which the gut receives its permanent blood-supply and in connection with the gradual reduction of the yolk-sac.

The study of the ventral branches of the aorta in the human embryo is based on the work of Mall, who in 1891 published an account of a human embryo 7 mm. long, in which he described two main ventral branches, a cœliac axis and an omphalo-mesenteric artery, and a series of small ventral branches in the lumbar region, making a capillary network in the mesentery. He noted that the position of both the cœliac axis and the omphalo-mesenteric artery was farther forward than in the adult, and analyzed all the available material in a study of the shifting of the arteries caudalward along the aorta. In this study he recorded human embryos with the cœliac axis opposite the first, second, fourth, and sixth dorsal nerves, as compared with the position in the adult opposite the twelfth nerve. In 1897 he made a further study of the ventral arteries, especially in a human embryo 2.1 mm. long. In this specimen he showed a series of ventral branches extending from the seventh somite to the caudal end of the aorta. These vessels he grouped together as the omphalo-mesenteric arteries. In the reconstruction he showed that the upper arteries tended to be opposite the middle of the somites rather than between the somites, as are the dorsal intersegmental vessels. In a second analysis of the ventral aortic branches he showed that there is a constant shifting of the cœliac axis and omphalo-mesenteric arteries caudalward. A double origin of the omphalo-mesenteric arteries in one embryo suggested the method of the wandering of the vessels.

The same idea of the shifting of the arteries caudalward was further developed by Tandler in two papers in 1904 and by Broman in 1908. These workers extended their observations over a long series of embryos, Broman giving a study of 41 specimens. In one of the youngest specimens in his series, an embryo measuring 3.4 mm., the upper ventral branch was between the sixth and seventh interspaces. He described the branches as tending to occur between the interspaces, there being two or three to a somite. He found that the cœliac axis and the superior mesenteric artery are not segmental vessels (that is, opposite the interspaces), while the inferior mesenteric artery is sometimes opposite and sometimes between the somites. Broman gives an analysis of the literature and an extensive discussion of the methods by which the shifting of the ventral arteries may take place.

In the human embryo ventral branches of the aorta have been described from about the seventh segment caudalward. In the pig these ventral branches are very numerous—approximately one to a segment and one to an interspace. They are originally of uniform size and about equidistant apart. They unite into an extensive plexus of larger vessels in the more cephalic region and into a long artery in the caudal region. It is easy to follow the method of the shifting of arteries from such a primitive pattern; that is, any of the vessels of the original system could easily enlarge and the blood-stream be increased or decreased according to the development of the region of the organ supplied. The entire wandering of the arteries can be understood without presupposing the development of any new vessels, but rather through the shunting of the blood through different channels already present in response to the varying development of the parts supplied by these arteries. Moreover, it is plain that the point brought out by Evans is of importance—namely, that the so-called wandering of arteries takes place while the vessels have the structure of capillaries; that is, while their wall consists of endothelium alone. From the position of the primitive ventral arteries it is also easily seen that there might be variations as to whether the ultimate ventral arteries of the older embryo came opposite an interspace on the same level as the dorsal arteries or opposite a somite.

## THE UMBILICAL VESSELS.

My series is not very complete in regard to the umbilical veins, but it shows a few interesting points. In plate 4, figure 3, the relation of the somatopleure to the fold of the amnion is very plain. In the somatopleure is the beginning of a capillary plexus representing the umbilical veins. In plate 5, figure 1, the umbilical veins are not injected, but they are well shown in plate 1, figure 1, in which it is clear that the return flow of the blood from the caudal end of the embryo is in part through the subintestinal artery in the splanchnopleure and in part through the umbilical veins in the somatopleure. At the stage of plate 1, figure 1, the umbilical veins have established their connections with the liver, though they still connect with the sinus venosus. In figure 1 of plate 1 and figure 2 of plate 5 it is clear that cephalic to the duct of Cuvier there is also a capillary plexus in

exactly the same position as the umbilical veins; that is, in the somatopleure. In the specimen of plate 1, figure 1, this venous capillary plexus connects with a tiny lateral aortic branch shown just opposite the zone of the second aortic arch. This lateral artery is not the second aortic arch, which arises from the ventral rather than from the lateral surface of the aorta. From this tiny lateral artery a straggling chain of capillaries is injected within the somatopleure, out over the heart, and down to the duct of Cuvier; they are omitted in the drawing. It is clear that they are vessels for the body-wall analogous to the vessels which drain into the umbilical veins; but they are cephalic to the duct of Cuvier. The venous end of the plexus is injected in plate 5, figure 2. It was shown in the chick that the corresponding vessel of the somatopleure over the heart develops very early. In later stages these vessels in the somatopleure over the heart anastomose freely with a plexus of capillaries lateral to the occipital myotomes, as shown in text-figure 5 in my article on the Origin and Development of the Lymphatic System, 1913.

## NEURAL BRANCHES OF THE AORTA AND THE PRIMARY HEAD-VEIN.

In connection with the neural vessels, I have no specimens of embryo pigs corresponding to the chicks of 6 somites in which to trace their beginning. I have one litter of very young pigs, measuring 3 mm., in which the heart and aorta are present; the neural folds are open at the cephalic end, and I can find no angioblasts along the closed hindbrain.

At the stage of figure 3, plate 4, the vessels to the forebrain can be injected; and the vessel of the hindbrain must be present, for it is seen in a human embryo of the same stage of development. Opposite the third and fourth somites the lateral plexus of the neural tube has been injected from the aorta. I found only one specimen of the litter of figure 3, plate 4; but the fact that the posterior cardinal vein is almost completely injected indicates that the anterior cardinal vein is present and that it connects with the deep vein of the hindbrain. At the stage of plate 5, figure 1, the vessels of the head are in about the stage of development of those of plate 1, figure 1, as is proved by the injections of the same litter. In one specimen of the same litter as plate 5, figure 1, the anastomosis of the capillaries around the optic stalk is complete, just as was shown by Evans for the later stage of three aortic arches in his figure 395 (Keibel and Mall, Manual of Human Embryology, II, p. 579).

The best view of the early neural vessels in my series is given in plate 1, figure 1. In order to analyze the relations of the vessels of the head, I have used gray to indicate all of the capillaries which are true neural vessels, in the sense of lying close to the wall of the neural tube and giving rise to the vessels of the subsequent pia mater.

As can be seen in plate 1, figure 1, the deep capillary plexus of the forebrain and midbrain is covering the wall of the brain, and the form of this plexus indicates the form of the brain. The vascular arch which surrounds the large peduncle of the optic vesicle (see Evans's figure 395) is incompletely injected in

plate 1, figure 1. It shows the relative size of the optic vesicle and the forebrain at this stage. The side of the thalamus and the midbrain is nearly covered by a plexus extending toward the dorsal wall of the neural tube. Along the cephalic part of the hindbrain is a wide vessel connected with the aorta by two arteries. It already shows sprouts along its dorsal border, two of which bound the otic vesicle. This deep single channel becomes a plexus along the side of the neural tube at a point just in front of the first myotome. This is the point where the cephalic end of the anterior cardinal vein joins the neural vessels, and, in terms of the neural tube, it is at the cephalic end of the origin of the roots of the vagus nerve. The transverse vessel of the first interspace which is so prominent in the chick is but a small vein in the pig like the other intersegmental veins, and does not become an important vessel, as in the chick. As is well known, the upper myotomes are occipital myotomes, so that it is clear that the point of transition between the deep vessel of the hindbrain and the primitive plexus, as shown in plate 1, figure 1, is not between the hindbrain and cord, but is near the upper part of the medulla. The lateral plexus along the cord is injected in the specimen of plate 1, figure 1, down to the fourteenth somite, which is opposite the lowest transverse artery injected, and the spinal arteries are injected down to the twentieth interspace. These lower vessels are omitted in the drawing.

In plate 1, figure 1, can be traced very clearly the origin of the cephalic part of the primary head-vein; that is, the primitive cerebral vein. Extending from the groove between the telencephalon and the diencephalon (as Evans showed in his figure 395 in 1912), is a superficial capillary plexus, indicated in blue, which receives its blood from the deep plexus of the forebrain and midbrain and drains into the deep vessel of the hindbrain. In this plexus will develop the primitive cerebral vein; at this stage it is entirely a plexus without any definite longitudinal channels. The specimen is just at the stage of the second vascular arch, which is probably present and uninjected, as shown in Evans's figure 394 from an earlier stage. Opposite the lower end of the primitive vessel of the hindbrain is a plexus of exceedingly tiny vessels spanning the gap between the deep vessel of the hindbrain and the anterior cardinal vein on the one hand, and reaching toward the second aortic arch on the other. These tiny capillaries form the origin of the lateral vein of the region, that is, the middle segment of the primary head-vein, just as has been shown for the chick. This plexus will span the gap between the second and third aortic arches as they form, and the cephalic end of the primary head-vein, until there is a double vascular channel from the head, as shown on plate 4, figure 1.

Figure 1 of plate 4 is from a specimen of the same litter as that of figure 2 of plate 5, and is given because of the extravasation in the head region in the latter figure. At the stage of three aortic arches the primary head-vein is complete. The primitive veins which pass ventral to the eye are not injected in the specimen of plate 4, figure 1, except just where they join the primary head-vein in front of the ganglion of the trigeminus. The primary head-vein starts opposite the thalamus and extends in a double curve down to the anterior cardinal vein. It

lies mesial to the Gasserian ganglion and lateral to the otic vesicle. The pattern of the deep and the superficial vessels in plate 4, figure 1, deserves careful study. The place of origin of the roots of the Gasserian ganglion is marked by a plexus of the deep vessels which are growing around it, leaving a non-vascular area where the nerves emerge. The deep plexus is also forming a dorsal arch around the otic vesicle which now lies between the deep and the superficial vessels. The pattern of the vessels also indicates the position of the acoustic complex of ganglia and the glosso-pharyngeal ganglion, both of which lie between the deep capillaries and the superficial veins, one cephalic to the otic vesicle and the other just caudal to it. It is clear that the relations of the primary head-vein to the Gasserian ganglion and to the acoustic complex are the same in the pig as in the chick, and are due to the fact that this vessel forms while the ganglia are attached to the skin in their respective placodes. The primary head-vein develops mesial to the placode of the Gasserian ganglion, but curves dorsalward opposite the acoustic ganglia and opposite the ganglion of the glosso-pharyngeus. Sections of an embryo slightly older than that of plate 4, figure 1, cut so that a long stretch of the primitive head-vein is included in one section, show that the lateral border of the acoustic ganglion is in a straight line with the mesial border of the Gasserian ganglion, so that the superficial vein takes the shortest course in passing mesial to the ganglion of the trigeminus and lateral to the ganglia of the acoustic complex.

The relations of the branches of the vena capitis prima are very important at the stage of plate 4, figure 1. The branches from the aortic arches are not injected, nor are the primitive maxillary veins. The lateral veins from the cerebrum have hardly begun. The superficial veins opposite the midbrain have a very characteristic pattern; they are, as it were, creeping along on the deep plexus toward the mid-dorsal line. It will be noted that the deep plexus itself has not yet reached the mid-dorsal line at this stage, but it is in advance of the superficial veins. This gradual extension of the branches of the vena capitis prima to the mid-dorsal line characterizes the branches of this vein over the entire brain. When the superficial veins meet in the mid-dorsal line they will give rise to all of the sinuses and veins of this line, as has been shown by Mall and Streeter.

Opposite the hindbrain the branches of the vena capitis prima have the same fundamental relation to the deep plexus. It is true that caudal to the otic capsule there are a few veins from the deep plexus draining into the ventral border of the vena capitis prima, which may be forerunners of the small ventral veins of the medulla in the adult, but almost all of the veins of the hindbrain drain into the dorsal border of the primary head-vein. These veins have the same characteristics as the rest of the neural veins; that is, they gradually creep dorsalward on the deep plexus. Over the hindbrain, however, the pattern of the veins is not as simple as over the midbrain, because here they are profoundly affected by the ganglia of the hindbrain and by the otic capsule.

It has been shown that the deep plexus makes an arch of capillaries around the roots of the nerves, as seen around the root of the trigeminus in plate 4, figure 1. The superficial veins also curve around the roots of the nerves. Their

beginning is shown in plate 4, figure 1. Here it is clear that branches of the primary head-vein are tapping the deep neural plexus around the root of the trigeminus.

Lateral to the acoustic complex and to the ganglion of the glosso-pharyngeus, the branches of the primary head-vein make a very extensive plexus. The superficial venous arch around the otic vesicle is just beginning in plate 4, figure 1. The veins around the trigeminus and around the otic capsule are exceedingly important, because of their ultimate relations to the basal sinuses of the dura. These vessels are shown in plate 4, figure 1, at the stage when they are scarcely more than capillary sprouts. They will be traced farther in the next figure.

The specimen of plate 7, from a pig which measures 6.5 mm. in oil, is given to emphasize the fate of the primitive vein of the hindbrain, to bring out the ventral artery that now extends the full length of the nervous system from the base of the optic cup to the tip of the tail, and to show the characteristic relations of the veins to the neural tube and its ganglia.

The injection of the specimen of plate 4, figure 1, did not bring out the ascending neural arteries as did a corresponding injection of the chick (plate 6), but the specimen of plate 7 shows that there is now a longitudinal artery which extends from the primary aortic branch to the brain opposite the subthalamus, along the ventral or ventro-lateral border of the neural tube to its caudal tip. This artery is an anastomosis between all of the neural arteries, both cerebral and spinal. As can be seen in plate 7, the carotid artery leads to an arterial plexus which covers the lateral surface of the subthalamus and gives rise to a cerebral artery passing dorsal to the eye. The plexus on the subthalamus anastomoses with the plexus of the opposite side in the mid-ventral line; it is tapped by a vein leading to the primary head-vein just cephalic to the maxillary vein. Opposite the groove between the thalamus and the midbrain the two plexuses on either side of the subthalamus gives rise to a single ventral artery which curves along the ventral border of the neural tube down to the level of the third occipital interspace, where the single median artery becomes an arterial plexus. From this point to the caudal end of the spinal cord there is a double line of capillaries, such as was shown by Evans in his figure 440 (1912). As Evans showed, this double capillary chain will give rise to the anterior spinal artery. The importance of this longitudinal neural artery, which gives rise to the circle of Willis, the basilar artery, and the anterior spinal artery, is obvious. The anastomosis of the arterial plexus of the subthalamus and the ventral surface of the cerebrum with the corresponding plexus of the other side across the mid-ventral line accounts for the anterior communicating artery of the circle of Willis. At the stage of plate 7 the longitudinal neural artery is supplied by the two carotid arteries, by direct arteries opposite the hindbrain, of which two are shown on the right side of plate 7, and by all the intersegmental arteries on either side. This artery is not supplied as yet by the vertebral arteries, which form later as an anastomosis between the upper intersegmental arteries.

The arterial plexus over the subthalamus leads into a finely meshed plexus which covers the entire cerebrum except a small area in the mid-dorsal line near the thalamus. This plexus is not shown in the drawing, but it has the same character as the plexus over the midbrain. The cerebral plexus completely surrounds the optic stalk; in this plexus the only vessel larger than the rest is the cerebral artery, which is seen dorsal to the eye in plate 7. The longitudinal neural artery along the ventral border of the midbrain and the hindbrain gives off a series of nearly equal, regular, small arteries which lead into the capillary plexus on either side of the neural tube.

The capillary plexus on the neural tube is very characteristic. As has been said, it is finely meshed over the cerebrum, the thalamus, and the midbrain; it is more coarsely meshed over the hindbrain, where the plexus has developed later, especially around the roof of the fourth ventricle, which has not yet been invaded by the vessels. The plexus on the hindbrain in plate 7 demonstrates the fate of the primitive vessel of the hindbrain, the beginning of this plexus as coming from the primitive vessel of the hindbrain having been seen in the living chick. The primitive vessel of the hindbrain disappears only in giving rise to the capillary plexus of the hindbrain. If the pattern of the neural plexus in plate 7 is observed carefully it will be seen that there is just a suggestion of transverse lines in the plexus, indicating that the direction of the flow of the blood is from the ventral to the dorsal border of the neural tube. In this plexus will ultimately come transverse arteries. Opposite the first somite will be noticed the beginning of three layers of vessels, a deep layer of very fine capillaries, a second layer of larger vessels also shown in gray, and a third layer of more superficial veins. This is the very beginning of the next stage in the development of the neural vessels.

The most important point about the form of the deep plexus on the neural tube is the way it conforms exactly to the neural tube and its nerves. Over the midbrain the plexus is very uniform, but over the hindbrain the character of the plexus indicates very clearly the position of the nerves. At the stage of plate 7 there are bare spots, that is, places with no blood-vessels, on the hindbrain corresponding to each nerve root; in later stages the vessels penetrate between the small bundles of the fibers of each root and then an injection of the deep plexus does not show the position of the nerves so clearly. As seen in plate 7, the positions of the roots of the trigeminus nerve and of the acoustic group of nerves are very clear. The otic capsule now lies just lateral to the deep capillary plexus, and thus its position is indicated only by the superficial veins. Opposite the ganglion of the glosso-pharyngeus is a bare spot in the deep plexus, which is nearly hidden by a very extensive group of superficial veins. The position of the roots of the vagus and the spinal accessory roots along the line of the posterior cerebral vein is very important. It is clear that the deep plexus outlines this long line of nerve roots, and the same is true along the more ventral line of the medulla, where the pattern of the vessels indicates the position of the roots of the hypoglossal nerves.

Along the spinal cord the pattern of the capillary plexus shows the position of the ventral nerve roots in the same manner.

The veins which form the branches of the vena capitis prima must now be followed. The veins from the visceral arches, still largely in the form of capillaries, are completely injected in the specimen, but are indicated in the drawing only at the point where they join the middle segment of the primitive head-vein. In plate 7 the cerebral and the cardinal segments of the vena capitis prima are shown in plastic form, but the middle segment is shown merely in outline in order to make more plain the relations of the neural artery beneath. Beginning with the maxillary vein, the entire maxilla is filled with a capillary plexus which leads to the maxillary vein. This capillary plexus anastomoses with the plexus of the mandibular arch. Besides these capillaries the vein receives a large group of tiny superficial veins which arise in the deep plexus that covers the entire olfactory area of the cerebrum, together with primitive ophthalmic veins which arise in the marginal vein of the optic cup, as in plate 6. One of these subophthalmic veins runs in the groove of the optic stalk. These cerebral veins from the rhinencephalon and from the inferior part of the eye are very important in the early drainage of the brain, but it is well known that the main permanent ophthalmic veins develop dorsal to the eye.

In the zone dorsal to the eye at the stage of plate 7 is a group of tiny superficial veins opposite the cerebrum which are like the small veins over the midbrain. They were omitted in plate 7, but are adequately shown for the chick in plate 6, and they are alike in both forms. These are the primitive cerebral veins. Over the midbrain the veins are characteristic. It is plain that they are lifted off from the surface of the neural tube, that they are all superficial to the deep plexus; they spread out like a fan from the primary head-vein and clearly extend along the deep plexus, which they tap at their tips, and approach the mid-dorsal line.

Opposite the hindbrain the veins are exceedingly interesting; they follow exactly the same general course of development as the rest of the neural veins; that is, they lie superficial to the deep plexus, are transverse to the long axis of the neural tube, and gradually extend toward the mid-dorsal line, constantly tapping the deep plexus at their tips. On the other hand, they are profoundly modified in their development of the ganglia of the hindbrain and by the otic vesicle, so that their pattern is much more complex than the pattern of those opposite the midbrain.

The vessels around the ganglion of the trigeminus deserve careful study. At this stage the entire lateral surface of the Gasserian ganglion is covered by a capillary plexus which was omitted in the drawing. This capillary plexus extends along the second and third divisions of the nerve and becomes continuous with the capillary plexus of the maxillary and mandibular processes. Besides this sheet of capillaries which covers the lateral surface of the ganglion, there are two transverse veins above and below the ganglion which outline the root of the trigeminus nerve. These veins are very characteristic, and mark the position

of the Gasserian ganglion in any injected specimen up to the stage measuring 20 mm., when the transformation of the veins into the dural sinuses is well advanced, as can be seen in Streeter's figure 3 (Amer. Journ. of Anat., 1915, XVIII, page 156). The superficial vessels around the ganglia of the eighth nerve are still in the form of capillaries in plate 7. Opposite the otic vesicle the deep plexus has completely covered the surface of the hindbrain; there are a few superficial veins across the lateral surface of the vesicle, which are shown cut off in the drawing close to the primary head-vein. Two of these transverse veins make a border for the otic vesicle exactly as do those above and below the Gasserian ganglion. In other words, the veins of the hindbrain can be most simply described as a series of transverse vessels, some of which are forced to curve by the Gasserian ganglion and the otic vesicle. Opposite the ganglion of the glossopharyngeal nerve is a series of transverse veins draining into the primary head-vein.

The veins opposite the vagus nerve are also very interesting. It is clear that the largest vein of the medulla at this stage is one which in a general way follows the roots of the vagus nerve. This vein was called the posterior cerebral vein by Mall. In general, the place where it joins the vena capitis prima marks the cephalic end of the anterior cardinal vein; it may be a single vein at its roots or a group of veins. In the pig the vagus nerve curves around its cephalic border, passing in the angle between this vein and the primary head-vein. Some of the injections show the nerve passing through a venous loop in this angle. Stracher describes the vagus nerve just caudal to the vein in the chick. The relations of the vagus nerve to the primary head-vein formed the basis of Kastchenko's original study of the primitive veins of the head.

As will be seen in plate 7, the main vein of the medulla primarily follows the course of the roots of the vagus nerve. It arches caudalward along the dorsolateral surface of the medulla in the line of the spinal accessory nerve and roots of the vagus. The line of the vein on the medulla can be well seen by following the vagus roots in Streeter's plate 11 (Amer. Jour. of Anat., 1905, IV).

While it is clear that this vein and its tributaries originally follow the path of the vagus nerve, if its development is followed it will be seen that it becomes a very important vein of the embryo, not even limited to the drainage of the neural tube. At the stage of plate 7 it anastomoses with the lateral venous plexus of the lower medulla, and the first and second occipital veins are correspondingly small. Subsequently it gives rise to an extensive group of dorsal branches that grow over the caudal part of the roof of the fourth ventricle and largely drain the developing choroid plexus. The posterior cerebral vein next develops an exceedingly interesting relation to the vascular system of the occipital myotomes. This relation was illustrated in two figures from injected embryo pigs in my article on the origin and development of the lymphatic system (1913, figs. 4 and 5). Opposite the entire zone of the myotomes a plexus of capillaries develops, forming the third vascular sheet of this region. Primarily there is a plexus of capillaries on the surface of the neural tube; secondly, a more lateral plexus of capillaries and veins especially related to the ganglia; thirdly, this sheet of capillaries lateral to

the myotomes. Opposite the occipital myotomes the capillary plexus drains, by a series of veins on the one hand into the main vein of the medulla, on the other hand into the anterior cardinal vein. The history of the neural branches of this vein of the medulla involves the entire subject of the circulation of the medulla. The relation of the branches from the occipital myotomes involves the subject of the development of the external jugular vein and its branches. The main stem of the vein was shown by Mall, in 1905, to become a part of the great transverse sinus. For this vein I am using the term *primitive posterior cerebral vein*. It might also be termed the primitive vein of the medulla.

The stage of plate 7 shows the beginning of the veins of the hindbrain. It will be seen that the primitive branches of the primary head-vein draining the hindbrain are greatly modified by the ganglia of the hindbrain and the otic capsule. Opposite the midbrain these veins are regular and nearly equidistant; opposite the hindbrain they are grouped according to the ganglia. Of these veins of the hindbrain, the group caudal to the Gasserian ganglion and the stem of the posterior cerebral vein bear the most important relations to the future cerebral sinuses at the base of the brain.

In this account of the early blood-vessels of the neural tube three facts have been brought out which are essential to an understanding of the development of the neural vessels. First, there forms a ventral neural artery, originally paired, which extends along the ventral surface of the entire neural tube from the base of the optic cup to the caudal end of the spinal cord, which is an anastomosis of all the direct neural arteries from the aorta; second, this artery leads to a capillary plexus which completely invests the neural tube and all its ganglia; third, the primary veins of the neural tube are all transverse vessels superficial to this primary plexus, and they gradually extend toward the mid-dorsal line and are profoundly modified by the ganglia, both cerebral and spinal. All of the veins of the brain drain into the primary head-vein. As has been shown by Mall and Streeter, the only segment of the vena capitis prima which remains as a part of the dural sinuses becomes the cavernous sinus, which is that portion of the primary head-vein medial to the Gasserian ganglion. All other dural sinuses develop from the branches of the vena capitis prima.

It has been shown that the middle segment of the vena capitis prima develops in the pig, as in the chick, as a chain of capillaries between the aortic arches and the anterior cardinal vein; it becomes very large, because it makes a more direct outlet for the primitive cerebral vein. The vena capitis prima develops from three segments and is the first true vein for the head; the primitive vessel of the hindbrain serves temporarily as a vein for the brain and then gives rise to the capillary plexus of the upper part of the hindbrain.

## CARDINAL VEINS IN THE PIG.

It was shown in the chick that the cardinal veins begin from dorsal diverticula of the aorta which project into the interspaces and dilate just opposite the dorsal border of the nephrotome. In the line of the nephrotome these separate dilatations become connected, making a common cardinal vein which, at the stage of 12 somites, is opposite every interspace. I have not the corresponding early stages of the cardinal veins in the pig. In my earliest stage in the pig, the cardinal veins are related to the aorta and to the spinal veins, as is shown for the chick in the section on plate 3, figure 2; that is, there are direct spinal arteries from the aorta to the cord and spinal veins leading to the cardinal vein. At the stage of plate 4, figure 3, the posterior cardinal vein is injected, extending from the zone of the ninth intersegmental artery almost to the duct of Cuvier. The anterior cardinal vein is not injected, but must be present in the specimen. The pig embryo shown in figure 1, plate 1, gives the best view of the cardinal veins in my series. In this specimen it is clear that the anterior cardinal vein joins the neural plexus cephalic to the first somite, so that the vein of the first interspace which was so important in the chick is like all of the rest of the intersegmental veins in the pig. Opposite the first nine somites in the pig, as shown in plate 1, figure 1, the cardinal veins appear to be an accompanying vein to the aorta. Just below the ninth intersegmental artery in the pig there are the lateral arteries to the nephrotomes, and over all of the rest of the course of the posterior cardinal veins the lateral cardinal vein must also be considered. Opposite the first nine somites I have not been able to find any direct connections between the cardinal veins and the aorta, such as were shown for the earlier stages in the chick. In other words, the cardinal veins are well formed rather than just beginning in all of my specimens. One embryo, of the same litter as the one in plate 1, figure 1, showed some tiny sprouts of the anterior cardinal vein opposite the second somite extending toward the aorta; sections, however, did not demonstrate any connections, and I could not prove that they were not the beginning of tiny veins that soon drain the pharynx.

The series of the pig embryos also does not show the origin of the duct of Cuvier, but the fact that it is made up of an extensive plexus is well shown in plate 1, figure 1, as well as its relation to the umbilical veins. Below the zone of the ninth somite the cardinal veins will be considered with relation to the vessels of the pronephros.

## NEPHRITIC VESSELS IN THE PIG.

The nephritic tubules in the pig receive an early and characteristic blood-supply. For the limit for the chick between the pronephros and the mesonephros I have followed Lilly, who regards the tubules as belonging to the pronephros down to the fifteenth or sixteenth somite (page 190). For the pig I have arbitrarily followed Felix's estimation for the human embryo (1912, page 762). He places the limit of the pronephros at the fourteenth somite. It will be seen in figure 3, plate 4, that just below the ninth intersegmental artery a series of lateral

arteries gives rise to a plexus which is ventral to the posterior cardinal vein, but which connects with it. The Wolffian duct intervenes between this plexus and the posterior cardinal vein. In figure 1 of plate 5, and figure 1 of plate 1, are given very characteristic views of these lateral arteries to the pronephros, and in figure 5, plate 3, is shown a ventral view of the arteries of the pronephros from a specimen of the same litter as that of plate 5, figure 1, but a little farther developed.

Figures 1 of plate 5 and 1 of plate 1 show a series of tiny lateral arteries beginning just below the ninth dorsal segmental artery. These arteries are about four to a somite, corresponding to the number of the nephritic tubules, and are connected by a tiny longitudinal artery close to the aorta and by a tiny lateral vein. In text-figure 4 is shown a section from a specimen of the litter of plate 1, figure 1, passing through about the fourteenth somite, showing an injection of one of these lateral arteries. Its exact position with reference to the developing tubule is, I think, important. This is most clearly recognized from the diagram given by Felix of the development of the nephritic tubules (fig. 561, Keibel and Mall, Manual of Human Embryology, page 804). The stage corresponds with diagram *d* of Felix's figure, and the artery passes directly across the curved bowl which makes the neck of the future Malpighian corpuscle. This is the earliest stage of the vessels of the nephritic tubules I have injected. As is seen in text-figure 4, the lateral vein, the vena cardinalis lateralis, lies ventral to the Wolffian duct, while the vena cardinalis posterior lies directly dorsal to the duct. The posterior cardinal vein is plainly shown in text-figure 4, but was not injected so far caudalward in any of my series.

FIG. 4.—Transverse section of an embryo pig of 23 somites, passing through one of the lateral arteries of the pronephros. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 5 and 6. The level of the section is shown by a line on plate 1, figure 1. The section is 20 $\mu$ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. ×115. *A. om.*, a. omphalo-mesenterica; *A. pr.*, a. of the pronephros which was injected from the aorta; *V. om.*, v. omphalo-mesenterica; *V. c. l.*, v. cardinalis lateralis; *V. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.

Text-figure 5 gives a very interesting section from the same series as text-figure 4. The level of the section is shown in plate 1, figure 1; it is about halfway between the level of the lowest transverse artery injected and the allantoic arteries. At this level the nephritic tubule is in the stage of Felix's figure 561*b*, consisting of a Wolffian duct and a mass of nephrogenic epithelium. Here, instead of an artery which can be injected, the section shows a chain of angioblasts running ventral to the nephritic tissue to the lateral cardinal vein, and other sections show similar chains of angioblasts connecting the aorta and the posterior cardinal vein.

These angioblasts are mesial to the nephritic tissue. This section is, I think, similar to the section in Evans's figure 416 from a human embryo of the same stage, namely, with 23 somites, which shows the posterior cardinal vein dorsal to the Wolffian duct and the lateral cardinal vein ventral to the duct.

FIG. 5.—Transverse section of an embryo pig of 23 somites, passing through one of the lower myotomes and the mesonephros, to show the position of the subintestinal artery and a chain of angioblasts which will form an artery of the mesonephros. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 4 and 6. The level of the section is shown by a line on plate 1, figure 1. The section is 20 $\mu$ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. ×115. *A. mes.*, a chain of angioblasts which connect the aorta with the v. cardinalis lateralis and which will form an artery of the mesencephalon but were not injected because they are still solid; *A. si.*, a. subintestinalis; *V. c. l.*, v. cardinalis lateralis; *V. c. p.*, v. cardinalis posterior.

A comparison of text-figures 4 and 5 seems to me to indicate that the primary arteries of the nephrogenic tissue are ventral to the nephrotome, but when the tubules are farther developed the artery crosses the neck of the tubule; in other words, the tubules grow ventral to the arteries.

The study of the embryo pig at the stage of 23 somites (as shown in plate 1, figure 1, and in the sections of text-figures 4 and 5), seems to me to indicate that the posterior and lateral cardinal vessels extend caudalward in connection with chains of angioblasts from the aorta which pass dorsal and ventral to the nephritic tubules in lines which are very plain in figure 5.

In figure 5 of plate 3 is shown a ventral view of the pronephritic vessels in a pig of 20 somites, in which it is clear that there is a tendency toward a grouping of the transverse arteries of the pronephritic tubules around segmental lateral arteries. For example, between the ninth and tenth spinal arteries there is one lateral artery giving off four branches; between the tenth and eleventh spinal arteries are two lateral arteries from the aorta, with three transverse branches.

FIG. 6.—Transverse section of an embryo pig of 23 somites, passing through the allantoic arteries to show that the primitive allantoic arteries are in the splanchnopleure. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 4 and 5. The level of the figure is shown by a line on plate 1, figure 1. The section is 20 $\mu$ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. ×53. *A. al.*, artery of the allantois; *C.*, cœlom; *V. al.*, vein of the allantois in the zone where the splanchnopleure, the somatopleure, and the amnion are fused.

The longitudinal artery shown in plate 3, figure 5, persists for some time in the pig and connects the glomerular arteries even after the arterial tufts of the glomeruli are well formed. As seen in plate 3, figure 5, the transverse arteries lead directly to a lateral vein, which in turn connects with the posterior cardinal vein. Moreover, as is shown opposite the tenth somite, the posterior cardinal vein has many direct connections with the aorta.

The next stage in the development of the circulation of the Wolffian bodies is the formation of the mesial cardinal vein. I have illustrated the position of this vein in two sections, one from an injected pig embryo of 30 somites, measuring 7 mm. before the caudal flexure has formed, and the other from an injected chick of 69 hours' incubation (text-figs. 7 and 8). The mesial cardinal vein lies ventral to the nephritic arteries, close to the aorta, in the angle between the root of the mesentery and the Wolffian ridge. The course of the mesial cardinal vein can be readily imagined in plate 3, figure 5, wherein it is noted that opposite the tenth and eleventh somites the posterior cardinal vein is in the form of a plexus, dorsal

Fig. 7.—Transverse section of an injected embryo pig of 30 somites, to show a typical cross-section of the vessels of the pronephros of the pig after the v. cardinalis mesialis has formed—that is, to show the pronephros with a central artery and three peripheral veins. The embryo measured 7 mm. after fixation and dehydration; it had no caudal flexure and was a little farther developed than the one on plate 5, figure 2. All the vessels shown were injected. The arteries are represented in black, the veins in white. The section is 50 $\mu$ thick and is unstained. ×53. *A. pr.*, artery of the pronephros which gives off capillaries to the tubules and extends to the v. cardinalis lateralis; *V. c. l.*, v. cardinalis lateralis; *V. c. m.*, v. cardinalis mesialis; *V. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.

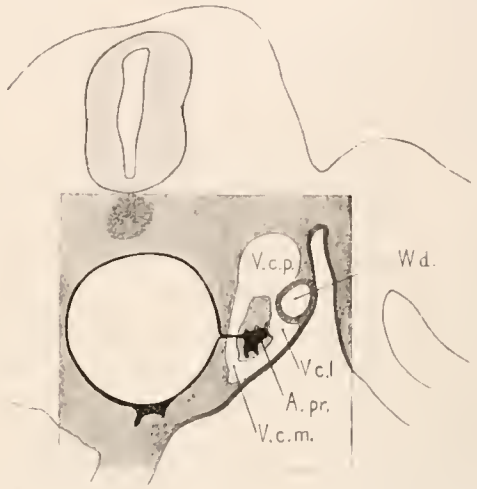


Fig. 8.—Transverse section of an injected chick of 35 somites, after 69 hours of incubation, passing through the fifteenth somite. The section shows a typical cross-section of the vessels of the pronephros in the chick after the v. cardinalis mesialis has formed—that is, it shows the pronephros with a central artery and three peripheral veins. All the vessels were injected. The aorta is shown with a black rim, the artery is black, and the veins are white. The section is 50 $\mu$ thick and is unstained. ×53. *A. p.*, artery of the pronephros; *V. c. l.*, v. cardinalis lateralis; *V. c. m.*, v. cardinalis mesialis; *V. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.

to the nephritic tubules (text-fig. 7). At the stage of 30 somites a vein from this plexus passes ventral to the nephritic artery opposite the eleventh somite and grows caudalward just ventral to the nephritic arteries, between the aorta and the longitudinal artery of plate 3, figure 5. This is the medial cardinal vein, the subcardinal vein of F. T. Lewis. There is thus formed the primitive pattern of the circulation of the Wolffian body, as shown in text-figures 7 and 8, consisting of a central artery and three longitudinal superficial veins—the posterior cardinal vein dorsal to the Wolffian duct, the lateral cardinal vein just ventral to the duct, and finally the mesial cardinal vein near the root of the mesentery. The mesial cardinal forms the connection with the vessels of the liver and (as shown by Hochstetter) also anastomoses with branches of the omphalo-mesenteric vein along the mesentery.

I emphasize the lateral cardinal vein because it has not been adequately recognized in the literature. In the pig it is very obvious in total preparations,

such as are shown in plate 3, figure 5. It develops early and is very straight. In the chick it is not straight and therefore is much less striking in total preparations. Its primary connections with the posterior cardinal vein are lateral to the Wolffian duct, as seen in text-figure 5 for the pig. That this is also true for the chick is shown by Graefe's figure 6 (1906), which shows the pronephros of the chick at the stage of 2 days and 15 hours. Later, in both the pig and chick, these two veins are connected by branches which are mesial to the duct, as shown in text-figures 7 and 8.

The failure to take into account the lateral cardinal veins has led to some confusion in the literature; for example, in the study of the pronephros, Graefe (in his figure 11) has labeled the lateral vein close to the Wolffian duct the subcardinal, while in figure 13 he has labeled the true subcardinal vein ventral to the nephritic artery the subcardinal, but has not labeled the lateral vein at all, though it is shown in the section.

In Keibel and Mall's Embryology, Felix gives some extremely interesting sections from the R. Meyer human embryo No. 300. This embryo had 23 somites and was 2.5 mm. long. It is to be compared with my plate 1, figure 1. In figure 532*a* Felix shows solid angioblasts, both dorsal and ventral to the Wolffian duct; he does not label the dorsal angioblasts which represent the posterior cardinal vein, but on the other hand calls the ventral angioblasts the posterior cardinal vein. Again, in figure 559 he calls angioblasts which are ventral to the duct the posterior cardinal vein. These sections show that in the human embryo there are angioblasts both dorsal and ventral to the duct and bring out the value of the two names for the veins, the posterior and lateral cardinal veins. They also show that the posterior and lateral cardinal veins extend as solid angioblasts and so bring up the question as to whether these veins may not differentiate as chains of angioblasts connected with the aorta by chains of angioblasts.

## CONCLUSION.

In this study it seems clear to me that the chick affords very valuable material for the study of the most fundamental point in connection with the vascular system that is still at issue, namely, how long in the life of the embryo do new angioblasts continue to differentiate from mesenchyme and join the blood-vessels? The answer to this question involves more extensive observations on the living blastoderm than I have yet made. It has been shown that blood-vessels first arise not only in the membranes but also in the embryo by a differentiation of cells into angioblasts, by the process which His had described, and not from a dilatation of spaces in the mesenchyme and a flattening-out of cells to form their border.

It has been proved that the aorta at least in part differentiates *in situ*. Evidence has been given that a part at least of the neural vessels and their connections with the aorta differentiate *in situ*. On the other hand, the cardinal veins begin as a growth from the wall of the aorta. They are a longitudinal anastomosis between direct branches of the aorta. A more detailed study of the later stages of the cardinal veins is necessary to determine if any part of them differentiates *in situ*.

I think that it is important to emphasize the extent of the development of the blood-vessels both of the membranes and of the embryo at the time when the circulation begins. This has been done for the chick, and it would be of great value to obtain the same observations for the mammal.

This study gives a more complete account of the primitive vessel of the hindbrain than is to be found in the literature. I have followed its origin, its relations, and its fate. The fate of this vessel is a very important point. This primitive vessel of the hindbrain differentiates early, opposite the first part of the neural tube to develop. It has been shown why it remains so long a single channel, namely, because it serves temporarily as a vein for the forebrain and midbrain before it takes the characteristic form of a plexus like the other early vessels on the surface of the neural tube. As the vena capitis prima becomes complete, so that the blood of the forebrain and midbrain is shunted out of the primitive channel of the hindbrain, this channel receives new arterial connections and breaks down into the very important capillary plexus of the rhombencephalon.

It has been shown that the first true vein of the head, the vena capitis prima, as contrasted with veins which drain only the brain, develops in three segments. The anterior segment is a purely cerebral vein which drains the forebrain and midbrain and originally empties into the primitive vessel of the hindbrain; the posterior segment is the anterior cardinal vein; the middle segment develops last, as a capillary chain between the capillaries of the maxillary, the mandibular and the other visceral arches, and the anterior cardinal vein. This middle segment anastomoses with the primitive cerebral vein from the forebrain and midbrain and forms a much more direct and favorable channel for draining the brain, and so rapidly supplants the more indirect channel along the hindbrain. It drains the other structures of the head in addition to the neural tube. The embryonic vein extending from the region of the thalamus to the duct of Cuvier is the first true vein of the head, in the sense of draining the entire head, that is, the brain and the visceral arches, and may thus be termed the vena capitis prima.

In connection with the vascular system of the nervous system, it has been shown that the early pattern of the blood-vessels is very uniform for the entire tube. There is a capillary plexus which completely invests the tube and all of its ganglia. It is fed by bilateral longitudinal arteries, which form as an anastomosis between all of the neural arteries from the aorta and extends from the carotid arteries at the base of the optic stalk to the tip of the spinal cord. The bilateral character of these arteries persists only around the subthalamus, where the circle of Willis is formed; elsewhere the two arteries become a single ventral artery—the basilar artery and its primary continuation, the anterior spinal artery. I have thus brought out the origin and the significance of the basilar and anterior spinal arteries and have shown that they precede the vertebral arteries.

The first neural veins are all transverse superficial vessels, which tap the deep plexus and gradually extend dorsalward on the deep plexus. They are profoundly modified by the eye, the ear, and by all the sensory ganglia. Opposite the brain they all drain into the primary head-vein; all the rest of the neural veins

are intersegmental branches of the cardinal veins. It is thus clear that the general direction of the blood to the neural tube is from the ventral to the dorsal border and that the direction of the flow of blood from the neural tube is the reverse.

In connection with the pig it has been shown that the large branches of the aorta near the caudal end of the embryo are primary allantoic arteries which run in the splanchnopleure, and that the umbilical arteries in the somatopleure develop later and anastomose with the primary allantoic arteries, exactly as in the chick. I have also given an analysis of the subintestinal vein of the chick and of the corresponding artery in the pig, and have shown that the fact that the vessel is an artery in the pig means that the primitive type of circulation of the yolk-sac persists longer in that form than in the chick. It has been shown that both the primitive allantoic arteries and the subintestinal arteries arise in a capillary plexus and end in a capillary plexus, so that in the case of these two vessels the blood must pass through two capillary plexuses in its return to the heart.

The study of the circulation of early embryos by means of injecting living embryos and watching the flow of the ink in them or by watching the circulation of the blood in the living specimen brings out some remarkable changes in the direction of the circulation; for example, the change in the direction of the circulation in the vessels of the area vasculosa in the chick when the veins invade a plexus which had been arterial. Again, in connection with the development of the primitive vessel of the hindbrain into a capillary plexus, the direction of the circulation is entirely changed. In the original vessel the blood flowed from the cephalic to the caudal border of the hindbrain, while when the new arterial connections bring blood to the entire ventral border of the vein the blood begins to flow from the ventral to the dorsal border of the hindbrain. In the case of the subintestinal artery is a third example of a profound change in the direction of the circulation. The blood originally runs through this artery out to the yolk-sac, but when the vessel becomes a capillary plexus in the wall of the gut, the blood flows toward the heart within the embryo in the new mesenteric veins.

From these studies it is clear that it is important to consider each vessel of the embryo from the standpoint of the function it performs throughout its development and that the effort toward a precise usage of the terms *artery*, *capillary plexus*, and especially of the term *vein*, is an effort to understand the circulation of the embryo.

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## EXPLANATION OF PLATES.

### Plate 1.

1. Injection of the vascular system of an embryo pig of 23 somites, which measured 7 mm. when fresh and 6.2 mm. after fixation and dehydration. The injection is nearly complete and shows especially the primitive relations of the vasa primitiva rhombencephali at the stage when it serves as a vein for the forebrain and midbrain and as a capillary plexus for the hindbrain. ×38. *A. l.*, artery of the first interspace; *al.*, allantois; *a. si.*, a. subintestinalis; *b. c.*, bulbus cordis; *l.*, liver; *pl.*, plexus on the spinal cord; *s. v.*, sinus venosus; *t. a.*, truncus arteriosus; *v. c. a.*, v. cardinalis anterior; *v. c. l.*, v. cardinalis lateralis; *v. c. p.*, v. cardinalis posterior; *v. u.*, v. umbilicalis; *va. p. r.*, vasa primitiva rhombencephali; *ven. c.*, ventriculus cordis.
2. Partial injection of the vessels of a chick of 12 somites. The needle was introduced into one of the omphalo-mesenteric veins near the heart. The transverse lines show the position of the interspaces. The sections shown in text-figures 1, 2, and 3 are from a chick of the same stage which was completely injected. ×54. *Me.*, mesencephalon at the level of the section shown in figure 1; *v. om.*, v. omphalo-mesenterica.
3. Injection of the heart and the cephalic aortæ, both dorsal and ventral, in a chick of 9 somites. The needle was introduced into the dorsal aorta opposite the somites. ×94. *Ao. d. c.*, aorta dorsalis cephalica; *ao. v. c.*, aorta ventralis cephalica ; *h.*, heart; *mc.*, mesencephalon; *v. om.*, v. omphalo-mesenterica.

### Plate 2.

1. Partial injection of the vessels of a chick of 14 somites. The needle was introduced into the dorsal aorta opposite the somites. The vascular plexus on the mesencephalon is not injected, though it is present at this stage, as is shown in text-figure 1. ×100. *A. so.*, artery of the somatopleure; *d. C.*, duct of Cuvier just before it has connected with the omphalo-mesenteric vein; *mc.*, mesencephalon; *v. c.*, v. cardinalis communis, that is, before it has an anterior and a posterior division; *v. so.*, vein of the somatopleure; *v. t.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
2. Injection of the blood-vessels of a chick of 16 somites, to show the relation of the primitive vessel of the rhombencephalon to the primitive cerebral vein, on the one hand, and to the anterior cardinal vein, on the other. This is the stage before the vena capitis prima is completed. ×58. *D. C.*, ductus Cuvieri; *v. c. a.*, v. cardinalis anterior; *v. c. p.*, v. cardinalis posterior; *v. ce. p.*, v. cerebralis primitiva, which will become the cephalic division of the v. capitis prima; *v. t.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
3. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-first interspace. The section is from the same series as figure 4 on the same plate, figures 2 and 3 on plate 3, and figure 2 on plate 4. Figure 4 on plate 3 is from another series. This section is to show the beginning of the spinal arteries as they show in the tenth to the seventeenth interspaces on plate 3, figure 1. The section is below the level of the omphalo-mesenteric arteries. It is 50 μ thick and is unstained. ×140. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.
4. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-seventh interspace. The section is to show the relative position of the direct arteries to the posterior cardinal vein and the arteries of the somatopleure. The section is below the level of the omphalo-mesenteric arteries and is in the region of the posterior limb-bud. It is 50 μ thick and is unstained. ×140. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.

### Plate 3.

1. The cardinal veins from an injected chick of 25 somites, to show the method of origin of the spinal arteries. ×106. *A. 3* and *a. 18*, arteries of the third and eighteenth interspaces; *d. C.*, ductus Cuvieri; *pl.*, plexus on the spinal cord; *v. c. a.*, v. cardinalis anterior; *v. c. p.*, v. cardinalis posterior; *v. t.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
2. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the fifteenth interspace. The section is to show a spinal artery like that of the seventh interspace of plate 3, figure 1; it is above the level of the omphalo-mesenteric arteries and shows the posterior omphalo-mesenteric veins on either side. The section is 50 μ thick and is unstained. ×140. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.
3. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the sixteenth somite. This is the next section in the series below that of plate 3, figure 2. It shows a direct dorso-lateral artery to the posterior cardinal vein. The section is 50 μ thick and is unstained. ×140. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.

## Plate 3—Continued.

4. Transverse section of an injected chick of 25 somites after 52 hours of incubation, passing through the seventeenth interspace. The section is to show the transition between the stage of figure 3 of plate 2 and figure 2 of plate 3, in the formation of a spinal artery. It is 50 $\mu$ thick and is unstained. ×140. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.
5. Injection of the aorta, the arteries of the pronephros, and the lateral and posterior cardinal veins in an embryo pig of 20 somites, from a specimen of the same litter as the one on plate 5, figure 1. The specimen is shown for the ventral aspect. ×140. *A. 9* to *A. 12*, arteries to the spinal cord in the ninth to the twelfth interspaces; *ao.*, aorta; *v. c. l.*, v. cardinalis lateralis; *v. c. p.*, v. cardinalis posterior.

## Plate 4.

1. Injection of the vessels of the head of an embryo pig of 27 somites, measuring 7.1 mm. after fixation and dehydration The specimen is from the same litter as the one on plate 5, figure 2, and is to show the completion of the vena capitis prima and its relation to the vasa primitiva rhombencephali. It shows that the primitive vessel of the hindbrain does not atrophy when the vena capitis prima is completed, but rather develops into a plexus on the hindbrain. ×94. *At.*, atrium; *b. c.*, bulbus cordis; *f. H.*, fretum Halleri; *t. a.*, truncus arteriosus; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment, which drains the forebrain, the midbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *va. p. r.*, vasa primitiva rhombencephali; *ven. c.*, ventriculus cordis; *ves. a.*, vesicula auditiva; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis; *IX*, position of the root of the n. glossopharyngeus.
2. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-fifth interspace. The section is to show the diverticula of the dorsal aorta like those of the first and second interspaces in plate 1, figure 2, which give rise to the cardinal veins. The section is 50 $\mu$ thick and is unstained. ×140. *Ao.*, aorta; *W. d.*, Wolffian duct.
3. Partial injection of the vessels of an embryo pig of 14 somites, measuring 4 mm. after fixation and dehydration. The specimen was injected through the dorsal aorta opposite the somites. ×56. *A. 9*, artery to the spinal cord in the ninth interspace; *at.*, atrium; *b. c.*, bulbus cordis; *f. H.*, fretum Halleri; *s. 1*, first somite; *s. v.*, sinus venosus; *t. a.*, truncus arteriosus; *v. c. p.*, v. cardinalis posterior; *v. om.*, v. omphalo-mesenterica; *ven. c.*, ventriculus cordis; *ves. a.*, vesicula auditiva.

## Plate 5.

1. Partial injection of the vessels of an embryo pig of 20 somites, measuring 6 mm. after fixation and dehydration. It shows the omphalo-mesenteric arteries, the subintestinal artery and the arteries of the pronephros; ×41. *A. 9*, artery to the spinal cord in the ninth interspace; *a. om.*, a. omphalo-mesenterica; *a. si.*, a. subintestinalis; *al.*, allantois; *b. c.*, bulbus cordis; *s. al.*, stalk of the allantois; *t. a.*, truncus arteriosus; *v. c. l.*, cardinalis lateralis; *ven. c.*, ventriculus cordis.
2. Partial injection of the vessels of an embryo pig of 27 somites, measuring 7.1 mm. after fixation and dehydration. It shows the general development of the vascular system at the stage when the vena capitis prima is completed. The vessels opposite the hindbrain, both deep and superficial, are extravasated in this embryo and hence they are shown on plate 4, figure 1, from an embryo of the same litter. ×51. *A. om. d.*, a. omphalo-mesenterica dextra, the other two omphalo-mesenteric arteries in the figure being on the left side; *at.*, atrium; *b. c.*, bulbus cordis; *d. C.*, ductus Cuvieri; *l.*, liver; *t. a.*, truncus arteriosus; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *v. om.*, v. omphalo-mesenterica; *v. u.*, v. umbilicalis; *ven. c.*, ventriculus cordis; *x.*, extravasation involving both the vasa primitiva rhombencephali and the vena capitis prima, as can be seen on plate 4, figure 1.

## Plate 6.

Injection of the vessels of the head of a chick of 29 somites, to show the origin of the vena capitis prima. The vein extends from the region of the dienecephalon to the duct of Cuvier. The injection shows that the vein arises in three segments; the first segment is a true primitive cerebral vein, which drains the forebrain and will soon drain the midbrain; the second segment is an anastomosis between the maxillary, the mandibular, and the other visceral arches and the anterior cardinal vein, and it drains the forebrain, the midbrain, and the visceral arches; the third segment is the anterior cardinal vein, which drains the brain and the visceral arches. ×128. *A. b.*, artery on the rhombencephalon, which at this stage is bilateral and is part of a plexus which will give rise to the basilar artery; *a. 3*, artery to the medulla in the third interspace; *d. C.*, ductus Cuvieri; *v. c. p.*, v. cardinalis posterior; *v. cap. p. 1*, v. capitis prima, first or cerebral segment which drain the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment which drains the forebrain, the midbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *v. m. p.*, v. maxillaris primitiva; *v. om.*, v. omphalo-mesenterica; *v. t.*, v. transversa of the first interspace; *v. u.*, plexus in which the v. umbilicalis will arise; *va. p. r.*, vasa primitiva rhombencephali; *ves. a.*, vesicula auditiva; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis.

## Plate 7.

Injection of the vessels of the brain of an embryo pig measuring 6.5 mm. in length after fixation and dehydration. The injection is a complete one, but the vessels of the visceral arches and most of the vessels of the cerebrum have been omitted in the drawing. The figure shows, first, the longitudinal artery of the central nervous system, which extends from the tip of the carotid artery to the caudal tip of the spinal cord; this artery is a plexus opposite the subthalamus, a single vessel down to the lower part of the medulla, and again a plexus on the cord; second, a part of the capillary plexus which invests the entire neural tube; third, the relation of the primitive veins of the forebrain, the midbrain, and the hindbrain to the vena capitis prima. ×73. *A. b.*, a. basilaris; *a. c. 1*, a. carotis interna; *a. 1*, artery to the medulla in the first interspace; *a. m. p.*, a. maxillaris primitiva; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment, which is shown only in outline, and which drains the forebrain, the midbrain, the hindbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein and which drains the brain and the visceral arches; *v. m. p.*, v. maxillaris primitiva; *ves. a.*, vesicula auditiva; *3*, *4*, and *6*, third, fourth, and sixth aortic arches, which are coming from the heart and are leading to the descending aorta, which is concealed by the cardinal segment of the vena capitis prima; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis; *IX*, position of the root of the n. glosso-pharyngeus; *X*, position of the root of the n. vagus; *XII*, position of the root of the n. hypoglossus.

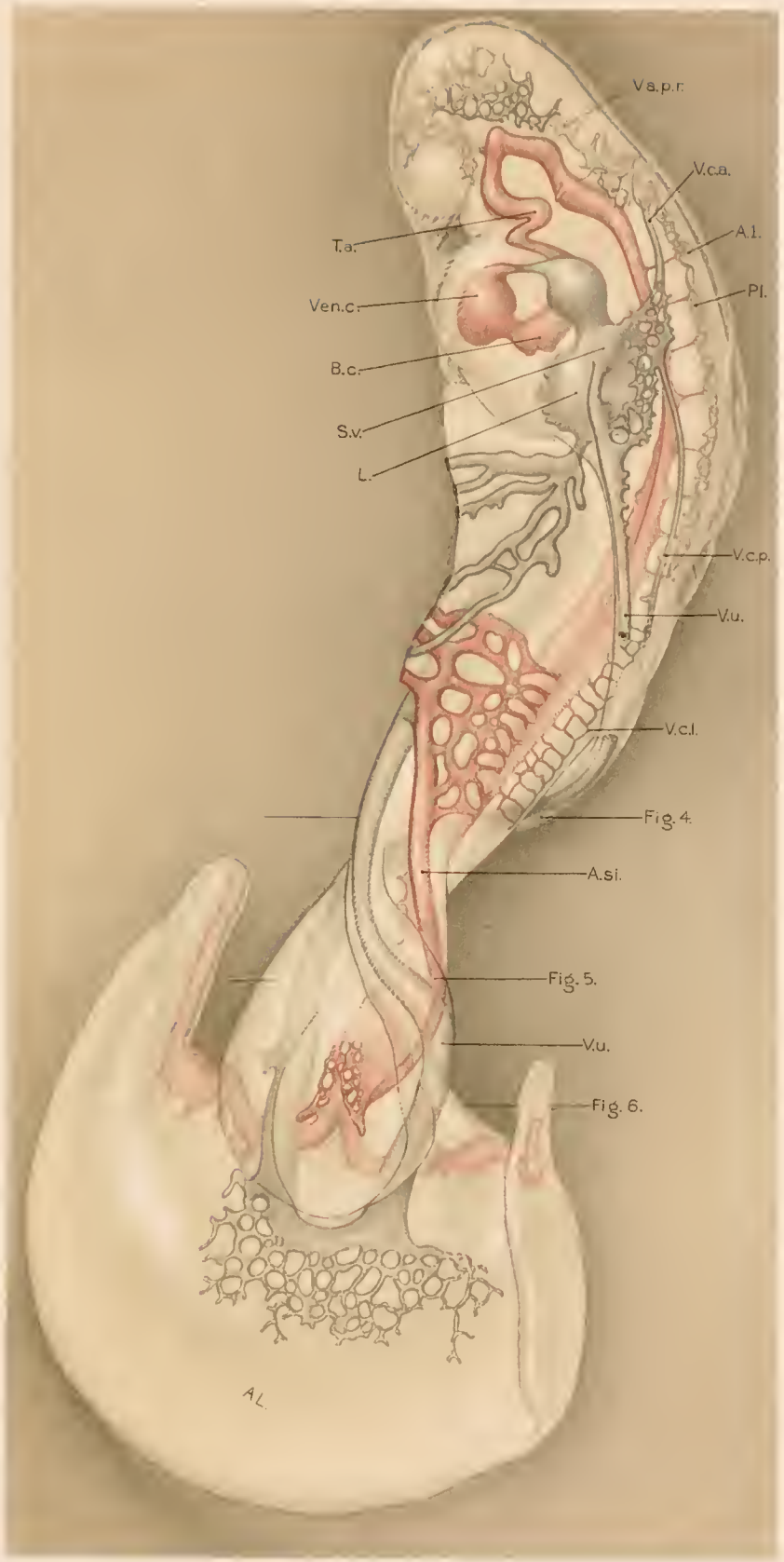



Fig. 1

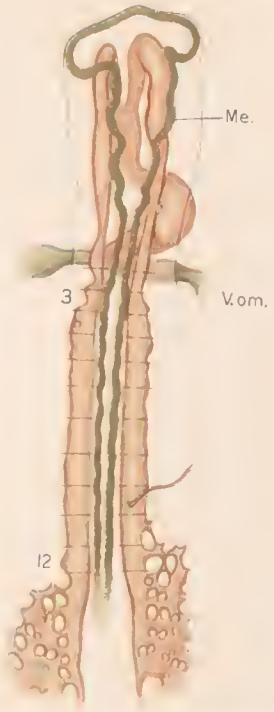



Fig. 2

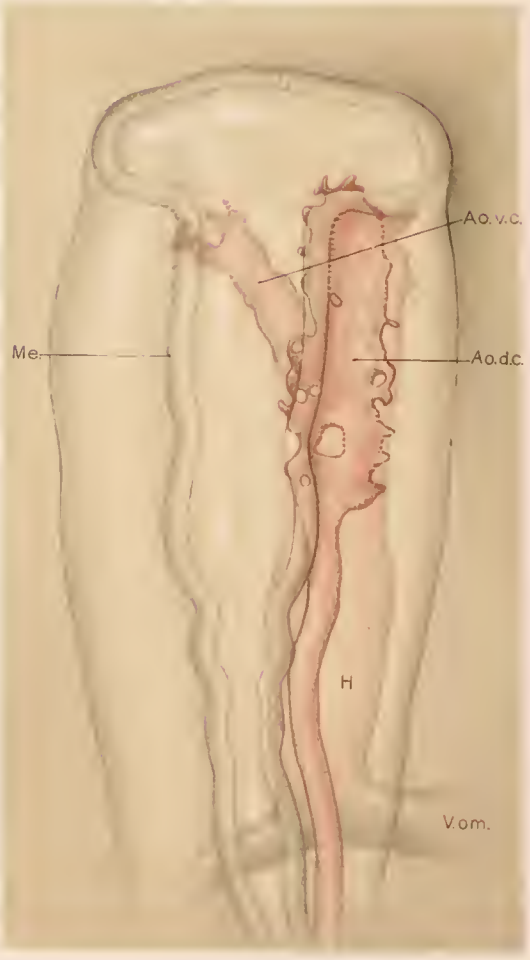
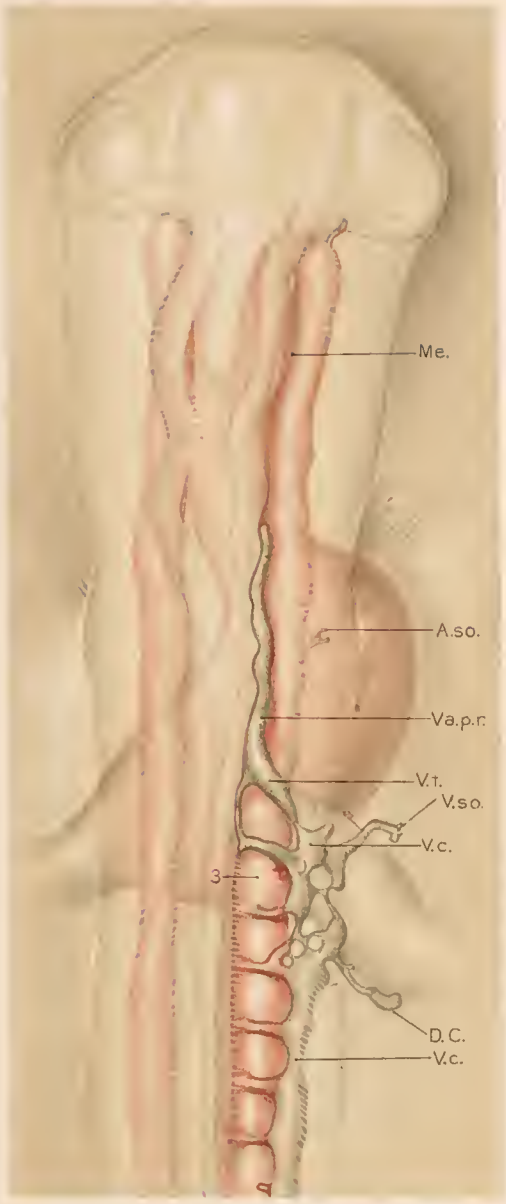



Fig. 3

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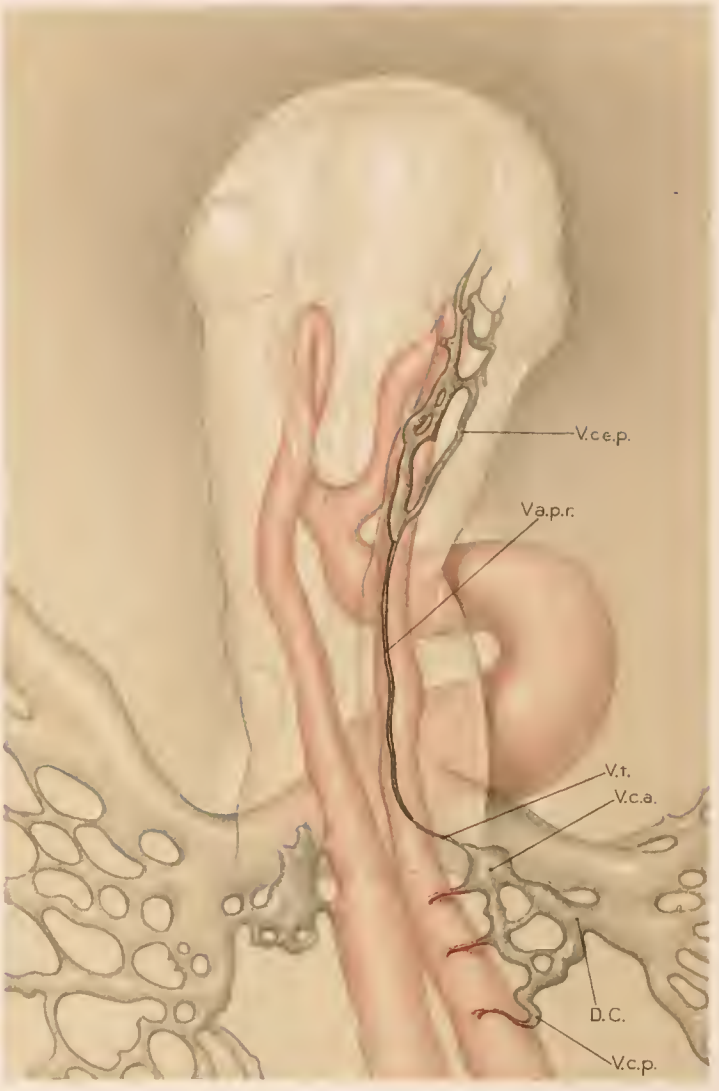


Fig. 2

Fig. 3

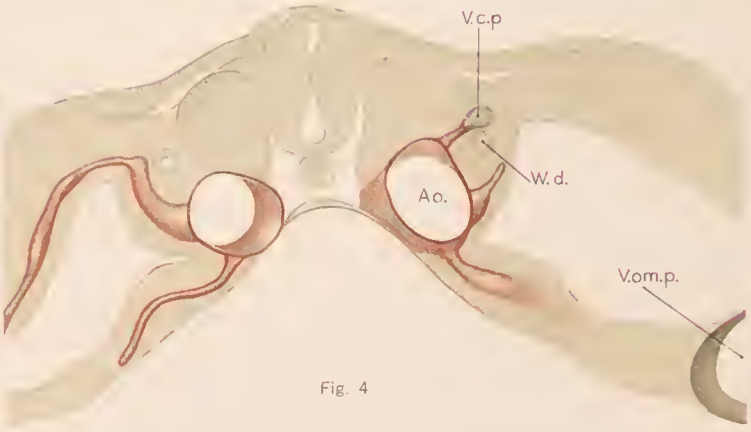


Fig. 4

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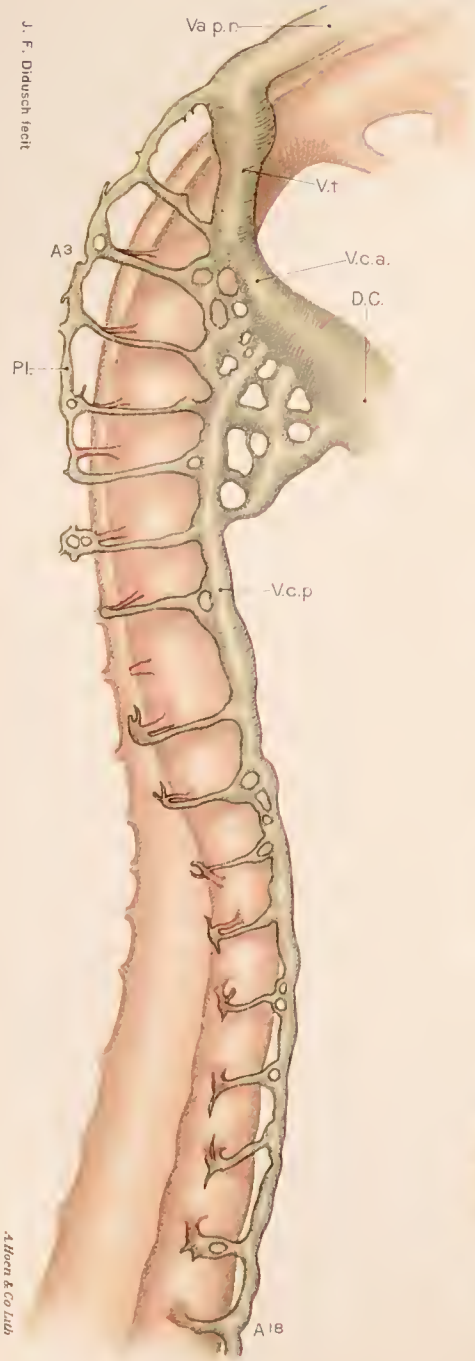


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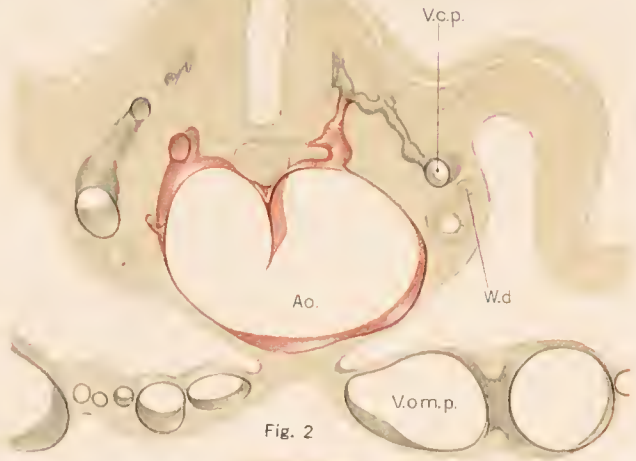
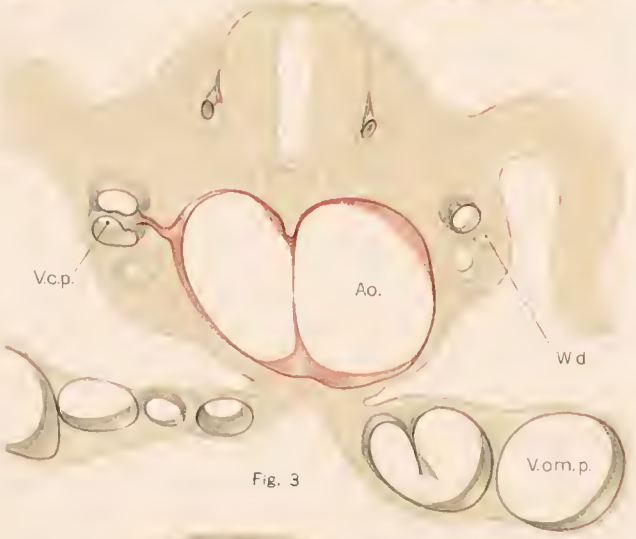



Fig. 2

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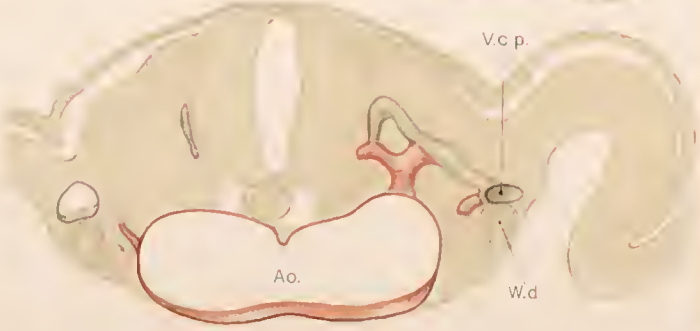


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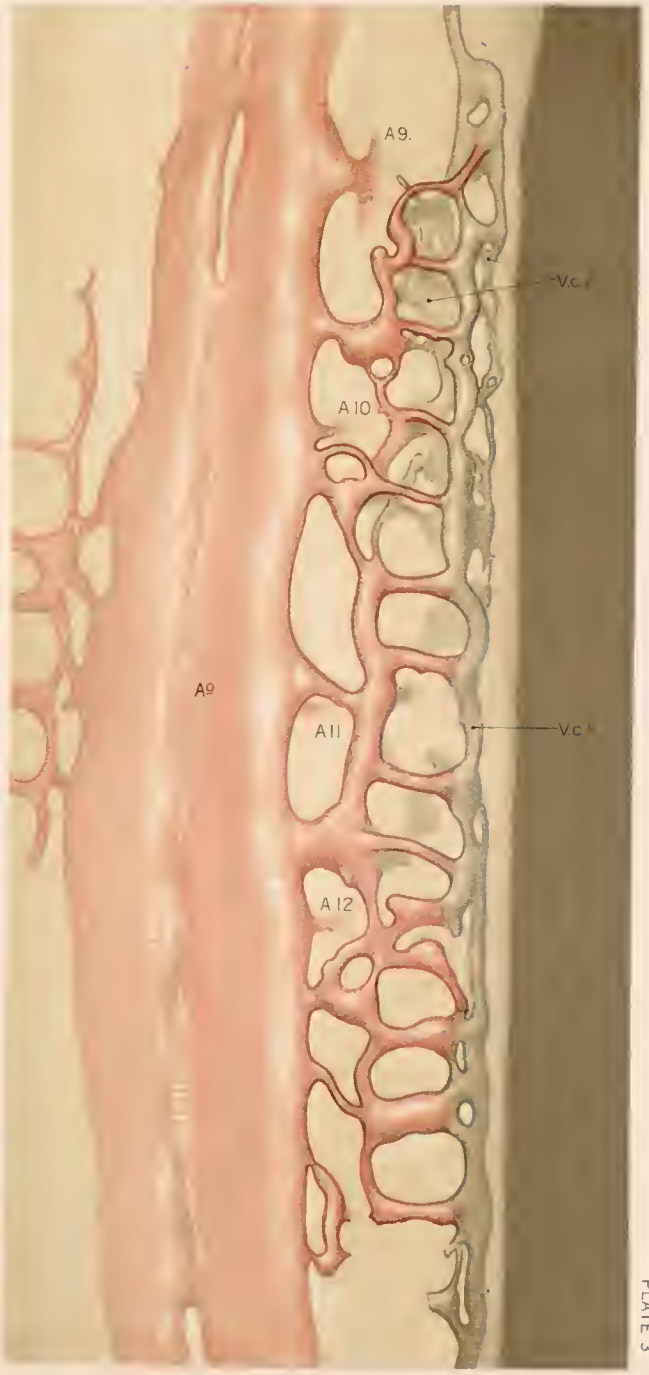


Fig. 5

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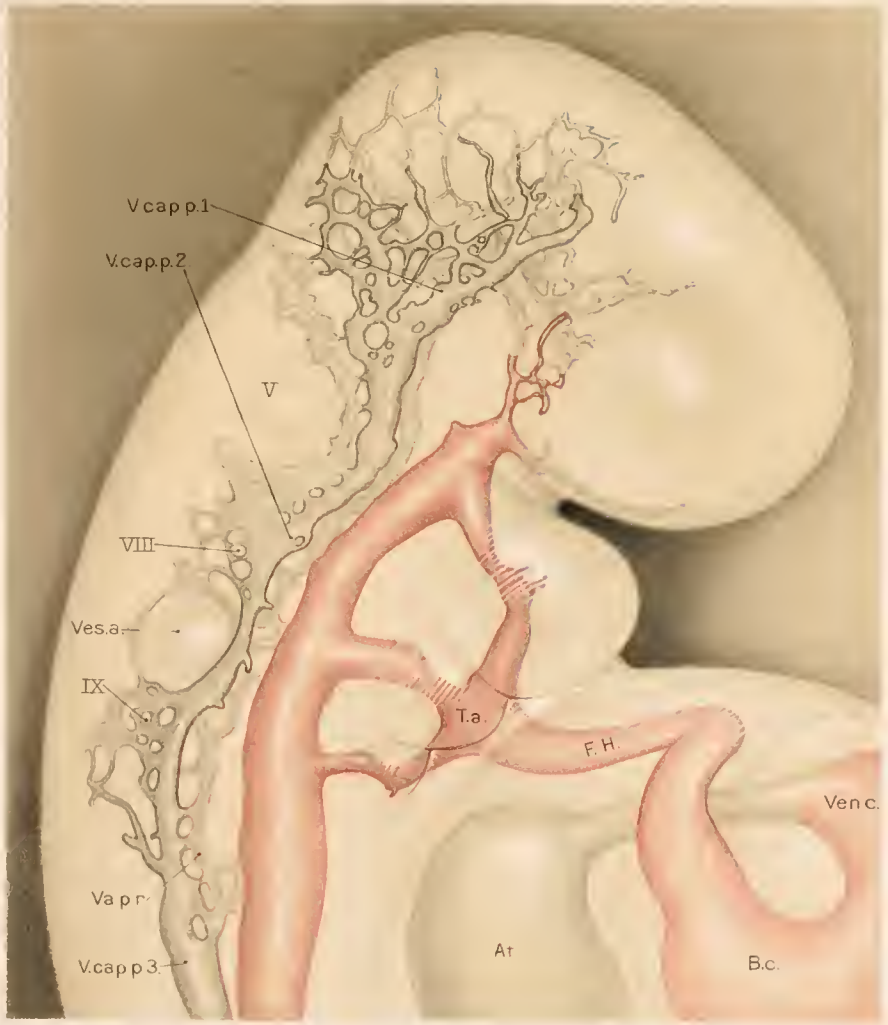


Fig. 1

Fig. 2

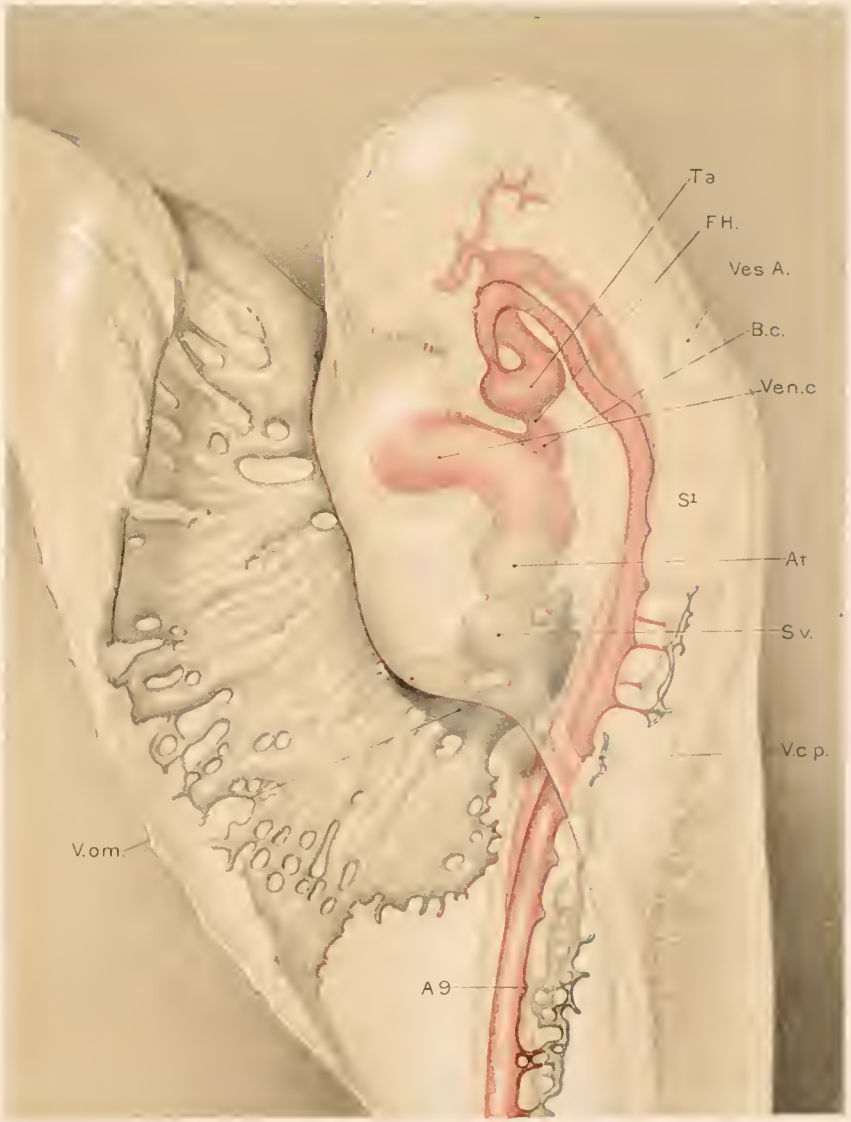


Fig. 3

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Fig. 1

Fig. 2

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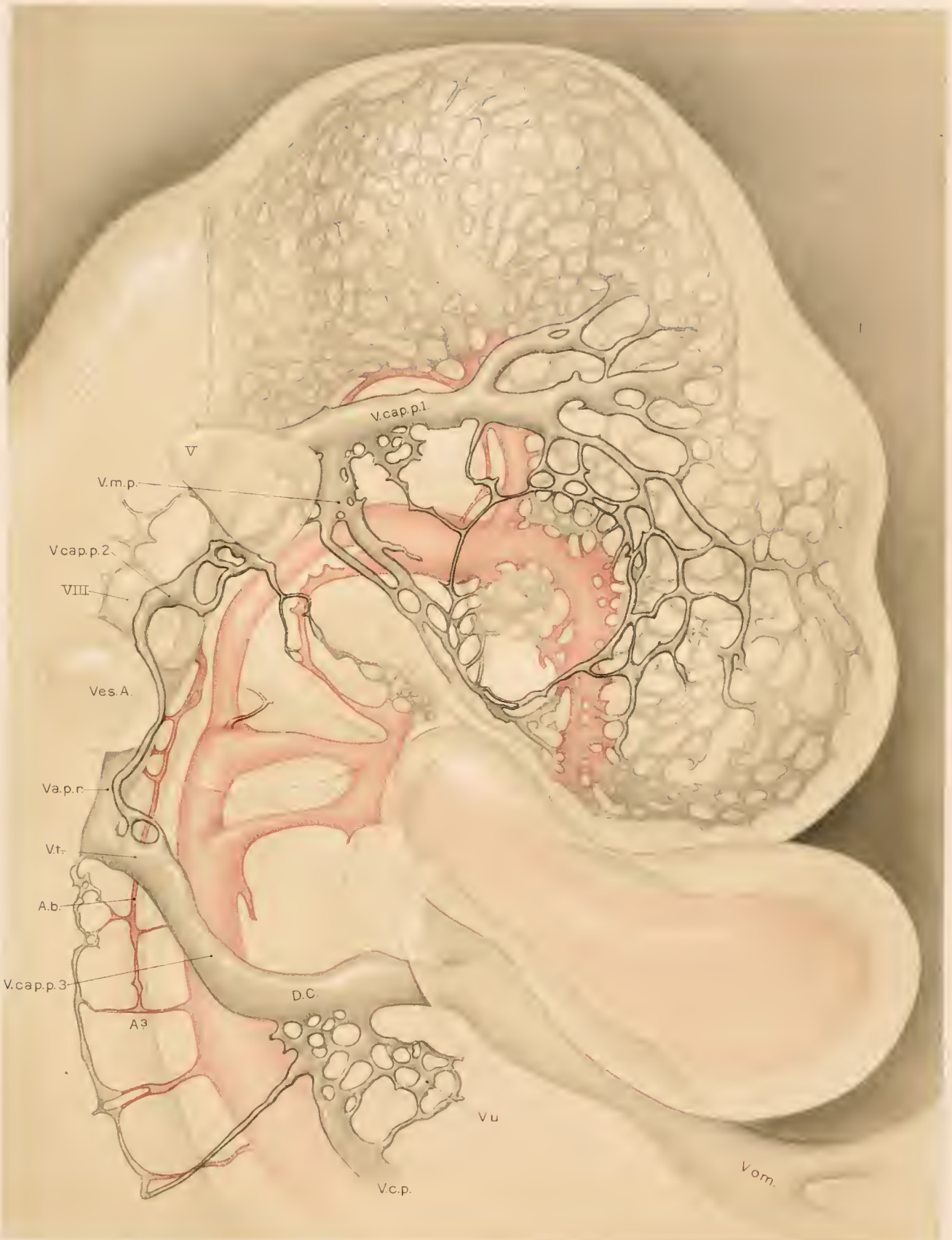


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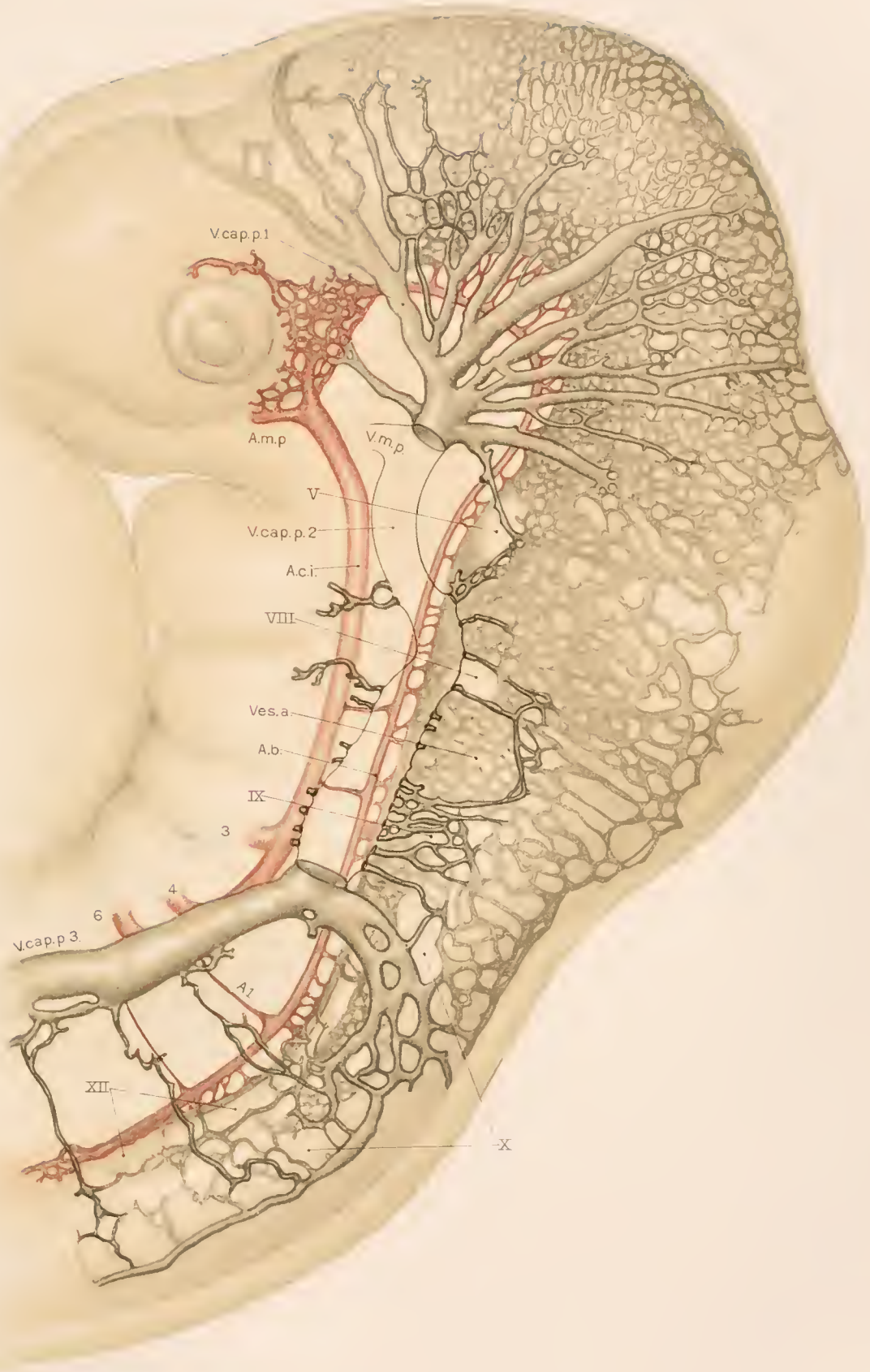


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CONTRIBUTIONS TO EMBRYOLOGY, No. 19.

# A HUMAN EMBRYO OF TWENTY-FOUR PAIRS OF SOMITES.

By Franklin Paradise Johnson,
*Of the University of Missouri.*

Eight plates, nine text-figures.

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# A HUMAN EMBRYO OF TWENTY-FOUR PAIRS OF SOMITES.

By Franklin Paradise Johnson.

## INTRODUCTION.

The embryo herein described was received February 9, 1914, from Dr. W. L. Allee, of Eldon, Missouri. Accompanying the specimen was a letter with the following information, but no further data regarding its history are obtainable:

The patient menstruated January 20 to January 25. Menstrual flow recommenced February 2, but it was freer and brighter in color than usual. On February 7 the specimen was aborted. Less than five minutes after abortion it was placed in 10 per cent formalin, in which fluid it was sent to me.

Fig. 1.—Drawing showing position of embryo in chorionic vesicle. Section 95 (of embryo). ×15 diameters.

The chorionic vesicle appeared as an elongated rounded body, of a cream color and a very delicate texture. It measured 15 by 9 by 8 mm. Extending from end to end of the vesicle, a flat fold with a slit in it was visible. It was covered with villi everywhere except along the fold. For fear of ruining a valuable embryo no attempt was made to open the sac. The whole vesicle was run

through the graded alcohols, cleared in chloroform, and embedded in paraffin. It was cut in sections 8 microns thick and stained with alum hemotoxylin and eosin. An idea of the plane of sectioning can be obtained from text-figure 1.

The age of the specimen is uncertain from the data obtained. As determined from a model of the embryo, its greatest length is about 2.4 mm. This measurement and those of the chorionic vesicle make it correspond very closely to an embryo of 28 days, as estimated by Mall[32], p. 199). If such is its age the specimen falls into that group of embryos which continue to develop in the uterus during a menstrual flow.

Comparison with descriptions of other embryos shows that my specimen is near the age of Robert Meyer's embryo No. 300, described by Thompson[45]; it resembles also Janosik's specimen[20] and His's embryo "Lg."[18]. In most respects, however, it is younger than the above-named specimens and also younger than the embryos described by Mall[29], Gage[12], Bremer[2], and Tandler[44]. It is apparently older than the twin specimens of 17–19 segments described by Watt[48].

A glance at the figure (plate 1, fig. 1) of the embryo is sufficient to show that there is a marked ventral bend in its back, such as has been found in many specimens of comparable age. Since this ventral flexure is not invariably present, and varies in degree, it is regarded by Keibel[22] as essentially abnormal. In my embryo it occurs without rupturing the underlying structures; if an abnormality, it is the only one which seems to be present.

I am indebted to my former students, Messrs. W. L. Brosius, L. B. Hohman, H. L. Houchins, L. H. Rutledge, Florien Vaughn, and T. F. Wheeldon, for certain reconstructions which have aided me greatly in my study of the embryo. To these men and also to Mr. G. T. Kline, who has made many of the illustrations, I wish to express my sincere thanks. I wish also to express my gratitude to Professor Frederic T. Lewis, who has read my manuscript and offered many valuable criticisms.

## EXTERNAL FORM.

The external form of the embryo has been studied from a wax reconstruction which was made at a magnification of 120 diameters. As already stated, the embryo was cut up without being previously drawn. It was necessary, therefore, to use the walls of the chorionic sac as guide-lines in making the model.

As seen from the left side (plate 1, fig. 1), the embryo is roughly the shape of a reversed S. Its back presents two well-developed curves. The upper of these is convex dorsally; it is large and rounded. The lower curve is convex ventrally, since the caudal portion of the embryo is bent sharply backward at about right angles to its longitudinal axis. Not only is the caudal end of the embryo bent backward upon itself, but at the point of bending it is twisted through an angle of 90° to the right. Thus the dorsal wall of this portion of the embryo is turned to the right and the ventral wall to the left; also, the caudal end of the embryo is directed to the left, so that its tip lies to the left of the axis of the body of the embryo.

The head of the embryo is large and rounded. When viewed from in front (plate 1, fig. 2), it appears somewhat egg-shaped. Toward its middle, on either side, are distinct outward bulgings, beneath which lie the optic vesicles. The mouth is a well-defined opening, limited on each side and below by the large mandibular arches.

Behind each mandibular arch there are three distinct gill-clefts. The first two of these are long and narrow and are directed obliquely to the longitudinal axis of the body, but the second bends dorsalward in its upper portion at almost right angles; the third is more shallow and rounded. The gill-clefts are bounded by four distinct arches. The first of these, the mandibular, is large and rounded, the second is similarly shaped but smaller, the third and fourth appear merely as rounded eminences.

The pericardium and heart lie just ventral to the arches. They form a large bulging which is more prominent on the right than on the left.

Beginning some little distance caudal to the last arch and placed at regular intervals throughout the remainder of the embryo, the mesodermic somites can be seen bulging through the skin. About 15 of these are perceptible from the surface, 10 in front and 5 behind the sharp flexure of the back.

The amnion is reflected from the embryo at the lower end of the pericardial cavity. The line of reflection here is curving and follows the lower curvature of the pericardial wall. At the side of the embryo, about halfway between the dorsal and ventral mid-lines, the line of reflection turns caudally, passes along the sides of the yolk-stalk, along the sides of the embryo, and finally on the body-stalk. Thus all the head, pericardial wall, and most of the caudal extremity and the dorsal portion of the remainder of the body of the embryo are within the amnionic sac.

Ventral to the line of reflection of the amnion is the yolk-sac. This is a large, irregular vesicle, broken through in places and flattened laterally. The yolk-stalk, which proceeds from the embryo just beneath the pericardial sac, is also flattened, but from above downward.

The outer surface of the hind gut, *i. e.*, its mesothelial surface, is distinctly seen turning backward to follow the curvature of the caudal extremity. At its bend there are two projections of the body-cavity, which likewise pass backward into the tail.

The body-stalk is attached to the embryo near its lower end, ventral to the caudal extremity. It turns sharply backward. The amnion is reflected from its upper surface for a short distance beyond the embryo. As a whole the body-stalk is short and broad, gradually becoming broader as the chorion is neared.

No indications of either fore or hind limb-buds can be found on the body of the embryo.

In comparing this embryo with Janosik's, it is seen that the back of the latter presents a more even curvature, which extends to the tail of the embryo. The angle formed at the top of the head (the midbrain bend) is almost identical in both specimens. The head of Janosik's embryo, however, is pointed, while in my specimen it is more rounded. In marked contrast to both of these specimens is the Robert Meyer embryo No. 300, as modeled by Thompson. Here the head

bend is very gradual, and the head itself much narrower and more pointed than in either Janosik's specimen or mine. Although somewhat similar in appearance to His's[18] embryos "Lg," "Sch," and "BB," my specimen differs from them in regard to the position of the ventral flexure of the back. In His's specimens the much-discussed bend in the back is always placed opposite the attachment of the yolk sac and stalk. In my specimen, however, the bend is placed further caudally, and the portion of the body which is bent backward is relatively shorter. The twin specimens which Watt[48] describes show definite ventral curvatures of the back, but these also are placed relatively higher up and are not as sharp as the bend in my specimen.

## INTEGUMENT.

In general, it may be said that the integument of the embryo is made up of one or two layers of ectodermal cells. The thickness of this layer and the shape and size of its cells, however, vary considerably in different regions of the body.

Over the sides of the head the ectoderm is thin, being composed for the most part of two layers of flattened cells with rounded nuclei. On the dorsum and front of the head the epithelium is still thinner, there being but one layer of flattened cells. Over the optic vesicles, where the lens placodes will later develop, there is as yet no indication of thickening, but in the region of the gill-arches the epithelium is considerable thicker, its cells being either cubical or columnar in shape.

In the region of the hindbrain there is seen from the surface a minute aperture (plate 2, fig. 1). Here the integument dips in and expands to form a sac, the auditory vesicle. This is flattened laterally, and approximately triangular in external view. It is closely applied to the brain, overlying the fifth and a part of the sixth neuromeres. With the exception of its form it is quite closely in accord with the more spherical vesicle of Thompson's embryo. The walls of the auditory vesicle are much thicker than the overlying ectoderm, and exhibit two or three layers of rounded or oval nuclei. Mitotic figures in the auditory vesicle are numerous.

The integument in the region of the mouth shows no especial thickenings. A few clusters of cells (the remains of the oral plate) are attached to it; anteriorly, one such cluster is found at about the level of the cephalic end of the notochord; other clusters are found on the sides and ventral wall of the oral cavity. The epithelium of the roof of the mouth is placed in close apposition to the floor of the forebrain, being separated from it by a few strands of mesenchyma only. There is no doubt that this portion of the oral integument is destined to become the anterior lobe of the hypophysis, but as yet there is no definite differentiation of this organ.

The integument of the body-wall of the embryo overlying the pericardial cavity is very thin, suggesting a stretching-out of the epithelium. It is also thin over the dorsum of the trunk and over the mesodermic somites. The integument which overlies the body-wall in the region of the umbilical vein is somewhat thicker and its nuclei are more closely packed together. It gradually thins out again as it is reflected to form the amnion.

## THE NERVOUS SYSTEM.

The nervous system is represented by the brain, with its two optic vesicles and the beginnings of the trigeminal, acustico-facial, glosso-pharyngeal, and vagus nerves, and the medullary tube with its ganglionic crest. The dorsal wall of the nervous system is placed just beneath the integument of the mid-dorsal line and conforms to all its curvatures. The cavity of the tube is entirely closed off from the outside, disregarding a longitudinal slit in the mid-dorsal line, which is clearly artificial, and in this respect differs from that of Janosik's embryo, which showed a small anterior neuropore.

### THE BRAIN.

The three primary vesicles of the brain are easily recognizable in plate 2, figure 1. The prosencephalon is large and bulbous, and is marked off from the mesencephalon by a deep groove. Broad in front and in the region of the optic vesicles, it gradually becomes narrower behind. Its cephalic end is rounded and lies in close contact with the ectoderm; an actual fusion is at one place apparent, marking probably the position of the closed anterior neuropore. There is no indication of a hemispheral division. The optic vesicles are attached to this portion of the brain slightly ventral and anterior to its middle; they extend outward, backward, and slightly dorsalward. There is as yet no definite indication of a division of the prosencephalon into diencephalon and telencephalon. A slight rounded protuberance of the ventral wall behind the points of attachment of the optic vesicles probably marks the beginning of the infundibulum.

The mesencephalon is a small wedge-shaped portion of the brain-tube. Of the grooves which mark it off from the prosencephalon in front and the rhombencephalon behind, the anterior is deeper; the posterior groove is faint dorsally, but ventrally it ends in a deep notch. The mesencephalon is much narrower from side to side than the prosencephalon. Its antero-posterior dimension is only a trifle greater than those of the neuromeres of the rhombencephalon immediately behind it. In marked contrast to this is the much longer and larger mesencephalon of the Robert Meyer embryo, as modeled by Thompson[45]. Somewhat similar to it, however, is that of Ingalls's embryo.

The rhombencephalon is elongated and flattened laterally. As seen from the side it is slightly curving, its dorsal wall being convex.

### MEDULLARY TUBE.

The spinal part of the medullary tube extends from the rhombencephalon to the tip of the tail, gradually tapering from above downward. It is ovoid in section, the lateral walls being thicker than the dorsal and ventral walls. The dorsal wall is thinnest and lies almost in contact with the covering ectoderm.

### NEUROMERES.

Both the rhombencephalon and spinal portion of the medullary tube are marked off by transverse grooves into a series of segments, the so-called "neuromeres." These begin at the cephalic end of the rhombencephalon and continue downward through the medullary tube. The first six neuromeres are narrow.

Lying next to the second neuromere is the ganglion of the trigeminal nerve; next to the fourth neuromere is the ganglion of the acustico-facial. The auditory vesicle lies opposite the fifth neuromere and partly overlaps the sixth; the latter is in process of giving off the cells of the glosso-pharyngeal ganglion. Beginning with the seventh, the remaining neuromeres are longer, being equal in length to the body segments. They do not lie within the segments themselves, however, but are arranged intersegmentally, the crest of each neuromere being placed opposite an intersegmental cleft. This arrangement begins with the first body segment and continues throughout the embryo. Above the first segment are $8\frac{1}{2}$ neuromeres.

The number of neuromeres belonging to the rhombencephalon can not be ascertained from this specimen alone. To determine this point, the author undertook a separate study of neuromeres based upon young human, pig, sheep, and cat embryos. Although this study is yet incomplete, it is evident that the last rhombic neuromeres stand out more clearly in certain specimens than in others; in some their presence is extremely doubtful. Just what this may be attributed to I am not able to state; it may be due to the stage of the specimen examined, or their presence on the one hand or absence on the other may be regarded as artificial. In those specimens which show distinctly a complete series of neuromeres, I have found that the first cervical ganglion is constantly related to the tenth neuromere. It appears evident, therefore, that the first 9 neuromeres belong to the rhombencephalon. It is to be noted that the first pair of somites in the embryo under discussion begins approximately opposite the crest of the eighth neuromere.

Of the previous description of neuromeres in young human embryos, those which appear to accord most closely with my own are the ones of Gage[12] and Watt[48]. In a description of an embryo of 28–29 pairs of somites, Mrs. Gage finds 9 folds or neuromeres in the rhombencephalon; of these the second is associated with the trigeminal nerve; the fourth with the auditory and facial nerves; the fifth is opposite the auditory vesicle; the sixth is in relation to the glosso-pharyngeal nerve; the seventh to the vagus; and the eighth and ninth to the accessory nerve. In regard to the spinal cord she states:

> "Beyond the clearly formed folds, above discussed, there occur several others, each corresponding with an enlarged part of the ganglionic cord. As this cord has no further indication of dorsal nerve roots, the exact relations can not be determined. Moreover, the following total folds in the myel (spinal cord) are not strongly marked, and in other specimens it is only in favorable sections that they can be seen at all." (pp. 435-436.)

Watt[48], in describing twin human embryos of 17-19 pairs of somites, similarly shows 9 neuromeres in the rhombencephalon. The tenth neuromere, he states, is opposite the first cervical ganglion. The results I have obtained with other mammalian embryos confirm this observation. Watt also shows spinal neuromeres extending along the medullary tube as far as the eleventh spinal ganglion.

In 1892, Minot[36] reviewed the earlier literature on this subject and made the general statement that "the entire medullary tube undergoes a segmentation by a series of alternating slight enlargements and constrictions." He adds:

"They appear first in the hind-brain and cervical region, and from there they appear progressively toward the fore-brain and the tail . . . . The medullary tube becomes slightly constricted between each pair of segments and slightly enlarged opposite each intersegmental space. Each intersegmental dilation is a neuromere . . . . Each neuromere produces a pair of nerves, but when the first trace of roots appears, they are seen to spring from the constriction between the neuromeres, but later from the neuromere."

Minot believes, therefore, that the so-called neuromeres of early stages represent, not true neuromeres, but the caudal and cephalic halves of two adjacent neuromeres.

An internal view of the brain is shown in plate 7, figure 5. The cavity of the prosencephalon is large and deep. Towards its anterior end the cavity of the optic vesicle is seen extending outward and backward. Posteriorily this is marked off from the forebrain cavity by a sharp indented ridge. The slight eminence of the ventral wall which will probably give rise to the infundibulum is again seen, being placed at the anterior end of the notochord. The walls of the rhombencephalon, when viewed from within, show the negative impression of the neuromeres, the "rhombic grooves" of Streeter[42].

The question of neuromeres in mammalian embryos is still an open one. Those of the spinal cord are undoubtedly related to the metameres, *i. e.*, representing true or parts of true morphological units of the medullary tube. Similar evidence concerning the "neuromeres" of the rhombencephalon is lacking. Although the arrangement of cerebral nerves is not contradictory to this view, *i. e.*, each neuromere, with the exception of the first, receiving afferent fibers and sending out efferent fibers (Johnson)[21], the muscles which the efferent nerves supply, the source of these muscles, and the "neuromeres," from which the efferent nerves spring, have not yet been demonstrated to coincide. In fact, the evidence, so far as gathered, contradicts this arrangement. Streeter[42] believes that the rhombic folds are not related to the metameric system, but is inclined to the view that "they may be fitted in with and form a part of the branchiomeric system."

He adds:

"The one discordant feature is groove *d* (5th neuromere), which has no corresponding branchial arch."

Neal[38], after extended observations and studies of head segmentation, in embryos of lower vertebrates, concludes that neuromeres offer no criterion for the determination of segmentation of the vertebrate head.

## CEREBRAL NERVES.

As in the Janosik and Thompson embryos, the ganglia of the trigeminal and acustico-facial nerves are clearly present. Of the two, the trigeminal arises a little higher dorsally than the acustico-facial. Each is made up of a cluster of nuclei which are more compact and deeply staining than the mesenchymal cells

which surround them. They are connected with the wall of the brain by strands of cells; definite fibers are apparently just beginning to form. Distally, ganglion cells and fibers can be traced for some little distance, the trigeminal nerve passing toward the maxillary and mandibular arches and the acustico-facial into the dorsal portion of the second arch.

In addition to the trigeminal and acustico-facial nerves, there is found just behind the auditory vesicle and near the brain-wall a small cluster of undifferentiated cells. These are found in the region of the sixth neuromere and very probably represent the ganglion of the glosso-pharyngeal nerve. Opposite the seventh neuromere is another similar group of cells, the probable beginning of the vagus nerve.

## GANGLIONIC CREST.

Indistinctly connected to the vagus ganglion and extending down beyond the ventral bend in the back of the embryo, a ganglionic crest is discernible. It is not distinct, however, for its cells so closely resemble those of other adjacent tissues that they can not always be identified with certainty. It is largely due to the position of its cells, *i. e.*, between the medullary tube and the somites, and to the arrangement of its nuclei, that the presence of the ganglionic crest can be detected.

Streeter[40] has described the neural crest of a 4 mm. embryo as follows:

> "This structure (the ganglionic crest) can be seen in the 4 mm. embryo as a flattened cellular band which extends caudalward from the auditory vesicle along the lateral wall of the neural tube to its extreme tip . . . . That part of the crest which corresponds to the spinal cord is characterized at this time by segmental incisures along its ventral border. The dorsal border of the crest remains intact until the appearance of the dorsal rootlets, in the meantime constituting a cellular bridge connecting the more ventral ganglionic clumps."

In my specimen the ganglionic crest likewise forms a cellular band. Segmental incisures along the ventral border are indicated in certain regions. The crest, however, is apparently not so far developed as shown by Streeter. Caudally its development is not so far advanced as toward the head. So indistinct and uncertain are its outlines, as seen in sections, that I have made no attempt to represent it graphically.

Lenhossek[25] has shown the ganglionic crest in a formative stage in a human embryo of 13 segments. Its cells are described as arising from the roof of the medullary tube. Such pictures as Lenhossek has shown I have been able to find in my specimen only in the caudal portion of the body, where the roof of the medullary tube is relatively thick; there, as seen in cross-section, the developing ganglionic crest appears as a cap lying on the roof of the medullary tube; it is composed of closely packed cells with nuclei of about the same size as those of the remainder of the tube.

## DIGESTIVE SYSTEM.

### MOUTH.

The mouth has been partly described in connection with the integument. It is directly continuous with the pharynx, the line of former separation being represented above by a few scattered clusters of cells, and below by a thin ridge of epithelium, remnants of the oral membrane. The oral cavity is broad transversely but narrow dorso-ventrally; there is no indication of the anterior lobe of the hypophysis. The scattered cells which Janosik[29] has designated as the hypophysis are undoubtedly remnants of the oral membrane.

I shall here mention His's[18] description of his embryo "Lg" (2.15 mm.), in which he recognizes both Rathke's and Seessel's pockets, while the oral membrane is still intact. Concerning these he says:

"Of the two peaked recesses between which it (oral membrane) passes, the anterior becomes Rathke's pocket, while posterior becomes Seessel's pouch."

In an embryo of 2.6 mm. in Keibel and Elze's Normentafel[23], in which the pharyngeal membrane is still present, the hypophysis is "just indicated." The Robert Meyer embryo of 23 segments, according to Thompson, shows no hypophysis, although the Normentafel states its beginning is "doubtful."

### FOREGUT.

#### PHARYNX.

The cavity of the pharynx is broader and deeper than that of the mouth. In the median plane its dorsal wall lies ventral to the notochord and follows closely the curvatures of that structure, being fused with it posteriorly (text-fig. 7). Ventrally the floor of the pharynx is more irregular. It possesses, toward its anterior end, a short, rounded diverticulum, the beginning of the thyroid gland (plate 2, figs. 2, 3, 4). This is in close relation ventrally to the ventral aorta, with which it lies in contact. Thompson describes a thyroid gland which is apparently in a similar stage of development, but Janosik and His (in his embryo Lg), fail to show this organ.

Behind the thyroid diverticulum the ventral wall of the pharynx shows two shallow depressions which cross the midline (plate 2, fig. 4). These are the cut sections of the transverse grooves (the "ventral pharyngeal grooves" of Grosser) which extend from side to side and connect the pouches of one side with those of the other. As described by Grosser[15], I find that the ventral groove of the first pouch before reaching the midline divides into two limbs which surround a median elevation. This he identifies as the "tuberculum impar." It is to be noted that the thyro-glossal duct proceeds from the summit of this elevation. This relation is noted by Grosser, who describes it as follows:

"The opening of the thyroglossal duct is situated at first upon the summit of the tubercle, but later it becomes shifted into the furrow bounding the tubercle posteriorly or, according to Ingalls, in an embryo of 4.9 mm., into 'the region of the second arch, immediately aboral to the tuberculum impar.' "

In a study of two embryos of 3 mm., Hammar[17] reports the tuberculum impar present in only one.

Between the ventral pharyngeal grooves of the second and third pouches is a second medial elevation in the form of a transverse ridge. From its position I believe it to be the so-called "copula" which, according to His and others, helps to form the tongue.

A third prominent elevation is found behind the ventral pharyngeal groove of the third pouch. It marks the point where the pharynx turns sharply caudalward. This probably is the area which Grosser had denoted the "cardiac swelling" and which he states goes into the formation of the larynx.

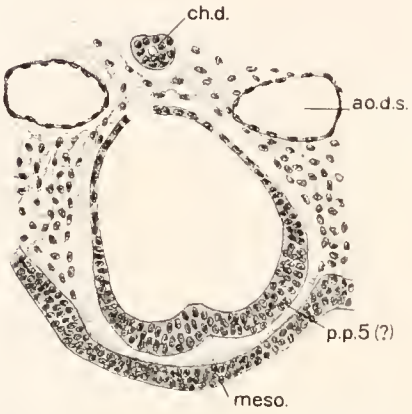


FIG. 2.—Portion of section 70. ×166 diameters.

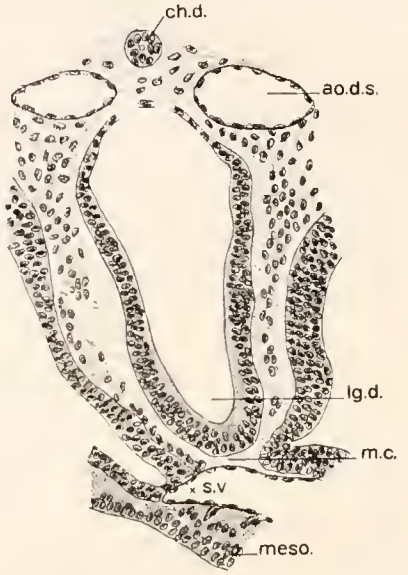


FIG. 3.—Portion of section 84. ×166 diameters.

The lateral borders or wall of the pharynx extend outward into three distinct pharyngeal pouches. Of these, only the first and second reach the ectoderm. The first pair of pouches is situated only a short distance behind the oral membrane. They are flattened from before backward, the left being somewhat broader than the right. In studying the first pouch of young embryos, Grosser[15] describes an invagination of its epithelium into the pharyngeal cavity as follows:

"In the region of the first pouch there projects ventrally or caudally from the closing membrane into the pharyngeal lumen an irregularly knobbed process filled with mesoderm . . . . It disappears quite early and may perhaps be interpreted as a rudimentary internal gill."

I have looked carefully in the region designated by Grosser for the structure which he describes, but have been unable to find any definite indication of it. A slight irregularity of the epithelium, however (more distinct on the left side than on the right), corresponds with it in position.

The second pouch is somewhat larger than the first and is flattened dorsoventrally. A distinct ventral diverticulum can be seen. The third pouch is more rounded in form and ends bluntly in the mesenchyma, falling somewhat

short of reaching the ectoderm. A fourth pouch is seen extending from the ventro-lateral surface of the pharynx. It arises to a certain extent in common with the third, and shares with it the third ventral pharyngeal groove. It is slightly pointed and is directed outward, backward, and downward.

The remaining portion of the foregut, that is, that part between the fourth pharyngeal pouch and the yolk-stalk, is shown in plate 3, figures 3 and 4. It presents, above, a definite swelling which is apparent when seen in either front or side view. Looked at from in front, the swelling appears double, a median longitudinal groove separating two rather elongated protuberances. A cross-section of this region is shown in text-figure 2. A short distance below this swelling there is another which, when viewed from the side, is seen to be rather pointed. It is just dorsal to the sinus venosus (text-fig. 3). Still farther caudally is seen the hepatic diverticulum (text-fig. 5).

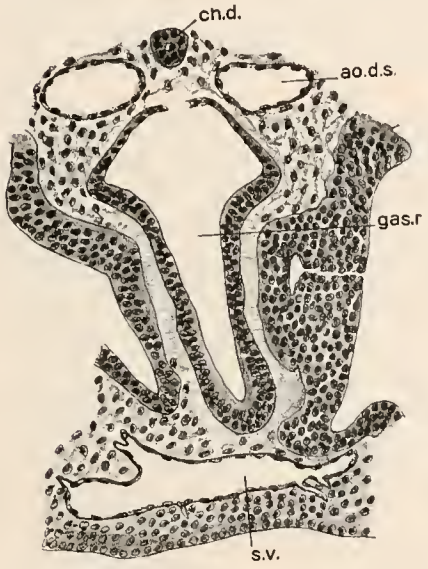


Fig. 4.—Portion of section 92. ×166 diameters.

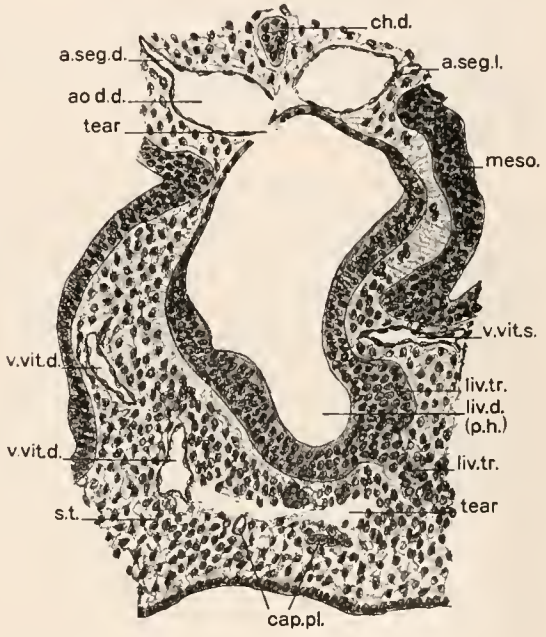


Fig. 5.—Portion of section 112. ×166 diameters.

## PULMONARY DIVERTICULUM.

The significance of the two upper swellings I am unable to determine definitely from this specimen alone. Thompson[45] describes a somewhat similar condition in the Robert Meyer embryo of 23 segments. The upper swelling he interpreted as a bilateral pair of lung-buds; the lower as the beginning of the stomach. Grosser[15], however, from a study of this region of the same embryo, concluded that Thompson's bifid swelling represents probably a fifth pair of branchial pouches, and that the lower swelling is the lung-bud.

Regarding Thompson's description he states:

"Thompson (1907) asserts that in this embryo the lungs have a paired origin, but he does not figure it, and this statement has been transferred to the Normentafel. But he is clearly in error as to the place where the lungs develop, as shown by his own descrip-

tion and a figure which he published later (1908). In 1908 he wrongly identified what is actually the lung-bud as the stomach, and 1907 he placed the lung-bud in the region of the diverticulum, which I have identified as a questionable fifth pharyngeal pouch. In fact, the embryo has as yet no indication of the stomach. Moreover, his model was probably made on too small a scale."

The longitudinal groove which Grosser[15] describes extending from the region of the last pouch down to the lung-bud is not present in my specimen.

According to Lewis[28], the Bremer embryo possesses a definite pyriform lung-bud, which is directed ventrally and caudally. The Broman embryo of 4.25 mm., as figured by Grosser[15], shows a similar lung-bud.

### GASTRIC REGION.

For the following unpublished statement concerning the gastric region of the Bremer embryo I am indebted to Professor Lewis:

"In the Bremer embryo a gastric region may be referred to, but it is not marked off in any way from the esophagus or duodenum. It may be located only by its relation to the liver and body cavities. It is a flattened or laterally compressed tube, having a cleft-like lumen."

From the condition found in the Bremer embryo, which is undoubtedly older than mine (having limb-buds), it would seem that the presence of a stomach in my specimen is improbable. A pulmonary diverticulum is to be expected. It seems to me, therefore, more reasonable to interpret the lower swelling, from its relation to the hepatic diverticulum, lying above the transverse septum and consequently in the pleuro-pericardial portion of the cœlom, as the lung-bud. The divided swelling situated above the pulmonary diverticulum probably corresponds to Grosser's doubtfully identified fifth pouches, but I am unable, from my specimen, to confirm Grosser's interpretation of its significance. That portion of the foregut between the lung-bud and hepatic diverticulum presumably corresponds to what Lewis[28] has designated the gastric region. A cross-section of this region is shown in text-figure 4.

### HEPATIC DIVERTICULUM.

A short distance below the gastric region the gut widens out considerably, forming a third diverticulum, the liver (text-fig. 5, and plate 3, figs. 3 and 4). It is directed ventrally and orally. It presents at about the junction of its lower and middle thirds a broad, shallow, transverse groove which divides the diverticulum into two portions, both entirely embedded in the septum transversum. Extending ventrally and laterally from the upper portion are a number of buds, the beginnings of hepatic trabeculæ; no such buds are found in connection with the lower part. The buds are composed of proliferating epithelial cells which, as indefinite cords, have invaded the mesenchyma of the septum transversum. Most of them are indistinct and their extent is doubtful, for their cells closely resemble those of the mesenchyma and there is no definite line of separation between the two, such as a basement membrane. They are readily overlooked, and it is only with an oil-immersion lens that they are made out with any degree of certainty.

Felix[9] notes the same difficulty in tracing hepatic trabeculæ in a slightly older embryo. Careful study shows that the nuclei of the entodermal cells are slightly larger than those of the mesenchyma, a point that is helpful in determining which cells belong to the trabeculæ.

The Bremer embryo (4 mm.) shows a somewhat more differentiated stage in the development of hepatic trabeculæ. Here they form anastomosing cords (Lewis)[28]. As shown by Ingalls[19] in a 4.9 mm. embryo, the trabeculæ are very extensive and form a large mass of anastomosing cords. Thompson[45], however, found no evidence of hepatic trabeculæ. In a later note[46] he states that "the transverse septum is seen before the cells of the liver bud have invaded the vessels which lie in it." He shows in sections, however, a transverse septum which, like the one in my embryo, is quite thick, and he apparently considers that all its cells (excluding endothelial and blood cells) are mesenchymal. Judging from the definiteness and size of the hepatic trabeculæ of the Bremer[2] embryo, which is evidently only a trifle older than Thompson's or my own, one would naturally expect to find some evidence of the trabeculæ in the latter two. I believe that, owing to their indistinctness, it is possible that they were overlooked by Thompson.

Regarding the development of hepatic trabeculæ, I believe it safe to draw the following conclusions: that they arise as indefinitely outlined buds of proliferating entodermal cells from the upper portion of the hepatic diverticulum; that while they grow in the mesenchyma and anastomose with one another, their cells undergo further differentiation and they become more distinctly differentiated from the mesenchyma.

I must mention briefly at this point the relation of the hepatic trabeculæ to the veins of the transverse septum. Janosik[20] has noted that the early hepatic diverticulum in the human embryo is not related to the vitelline veins in the same way as in birds. Bremer[2] states:

> "The liver cords are found growing into the mesenchyma, at a level ventral to the vitelline veins; in this same mesenchyma, however, we find the branches of the vitelline veins ramifying and forming plexuses, and in certain places these plexuses come into intimate relation with the liver cords."

I find with Janosik and Bremer that the hepatic diverticulum and trabeculæ are not in close relation to the vitelline veins. These lie dorsally and laterally to the diverticulum. Somewhat ventrally and anteriorly is found the sinus venosus. In the region of the hepatic trabeculæ can here and there be made out minute spaces which contain one or two red blood-corpuscles, apparently blood-vessels; but in my specimen I have been unable to make out a definite plexus as found by Bremer.

The lower knob-like portion of the hepatic diverticulum presents no special feature other than a very thick ventral wall. Brachet[1], in a careful study of the development of the liver in several different vertebrates, shows that in the rabbit the hepatic diverticulum is an elongated outpocketing of the foregut,

extending from the region of the sinus venosus to the yolk-stalk, and divisible into cranial and caudal portions. The upper of these he states goes into the formation of the liver proper and the hepatic duct; the lower into the formation of the gall-bladder and the cystic duct. The two portions are designated by Maurer[35] the "pars hepatica" and "pars cystica" respectively. The twin embryos which Watt described are apparently too young to show these divisions of the liver—in fact, the liver forms merely a slight swelling on the ventral wall of the foregut where the latter joins the yolk-stalk. It is called by Watt the "liver bay." Thompson, however, recognizes the hepatic and cystic portions of the liver diverticulum in the Robert Meyer embryo No 300. Somewhat similar divisions are described by Ingalls in his embryo of 4.9 mm., but his specimen is considerably older than the above-mentioned ones. Evidence of a division into two portions is apparently altogether lacking in the Bremer embryo of 4 mm., the liver diverticulum of which has been modeled by Bremer and more recently by Lewis.

## YOLK STALK AND SAC.

Just below the hepatic diverticulum the gut becomes narrow, but a little more caudally it again gradually broadens. This broadening leads out into the cavity of the yolk stalk and sac. The yolk-stalk is short, being in fact merely the constriction between the yolk-sac and the gut. It is flattened antero-posteriorly, but is broad transversely (plate 1, fig. 2). It measures roughly 0.5 mm. from side to side and 0.16 mm. antero-posteriorly.

The yolk-sac is a flattened vesicle, rather irregular in form, and with a number of folds of various shapes and sizes on its surface. It fills up practically the entire space between the embryo and the wall of the chorion, and extends into the artificially made fold of the chorionic wall as described above. Its dimensions are roughly as follows: length, measured parallel to long axis of embryo, 3.3 mm.; width, measured parallel to dorso-ventral axis of embryo, 2.7 mm.; thickness, measured transverse to embryo, 1.1 mm. Its histological structure will be considered later.

## HIND-GUT AND CLOACA.

Caudal to the place at which the yolk-stalk passes out is the beginning of the hind-gut. It has a funnel-shaped opening which tapers as it passes toward the tail into a small rounded tubule. It is surrounded by loose mesenchyma, the whole being attached to the dorsal body-wall by a short, thick mesentery. The hind-gut occupies a position slightly to the left of the median plane of the embryo. It bends backward with the body of the embryo at the ventral bend in the back. It passes without sharp demarcation into the cloaca (text-figs. 8 and 9). The cloaca is the cephalic portion of the spindle-shaped termination of the hind-gut. Its cephalic limit is not definitely indicated, but its caudal extent is marked by the cloacal membrane.

### ALLANTOIC DUCT.

The allantoic duct is a very long, slender, hollow tube which proceeds from the cephalic end of the cloaca and extends into the body-stalk. At its origin it is funnel-shaped, and its lumen is distinct. It tapers rapidly as it enters the body-stalk. For a few sections it becomes almost lost from view, owing to the indistinctness of cell boundaries and scattered nuclei. Although continuity of the allantoic cells can be made out, its lumen is lost. A few sections farther distally the allantoic duct again becomes distinct and a trifle larger. Lying between the two umbilical arteries, it follows the ventral bending of these vessels. Still lower down the arteries fuse and then split apart again, thus forming an arterial fork. The small allantoic duct passes in front of the fused part, and then, turning dorsally, passes through the above-described fork. Crossing the umbilical stalk obliquely, it terminates in a small bulb, the allantoic vesicle, which is situated close to the fused umbilical veins.

### CLOACAL MEMBRANE.

A short distance from the end of the gut is a very slight outward bulging of the ventral wall. This portion of the cloacal wall is in contact with the ectoderm of the proctodeal invagination, and together these layers of epithelium form the cloacal membrane (text-figs. 8 and 9). The entodermal portion is slightly thicker than the ectodermic.

### CAUDAL INTESTINE.

The portion of the gut beyond the cloacal membrane ends bluntly in the extreme end of the tail, separated from the ectoderm by only a small amount of mesenchymal tissue. This portion of the gut represents the post-anal or caudal intestine (text-figs. 8 and 9).

## HISTOLOGICAL STRUCTURE OF THE DIGESTIVE TUBE.

Histologically considered, the digestive tract may be described as an epithelial tube surrounded by mesenchyma. Only the former shows signs of differentiation as yet, the mesenchyma being everywhere of the same character. The epithelium takes on widely different appearances in different regions. In general it may be said that throughout the whole of the digestive tube, with the exception of the lower end, the dorsal wall is much thinner than the ventral. The former is made up of a single layer of cubical or flattened cells. On either side of the mid-dorsal line the epithelium gradually becomes thicker and the nuclei more crowded. The side-walls and floor of the pharynx show from two to three layers of nuclei. At the places where the entodermal epithelium of the pharyngeal pouches comes in contact with the ectoderm, it is thin and fused to the ectoderm. The membrane closing the second gill-cleft on the left side has broken through to the outside; this is undoubtedly a mechanical tear. The wall of the thyroid diverticulum is not different from that of the floor of the pharynx, being composed of an epithelium of two to three cell-layers. The epithelium of the ventral wall of the remainder of the fore-gut is thicker, the change from the thin dorsal wall to the

thick ventral wall taking place gradually on the sides of the tube. The pulmonary diverticulum is two to three cell-layers thick, the hepatic diverticulum three to four.

In the region of the yolk-stalk the epithelium is made up of one layer of cubical or somewhat flattened cells. In the yolk-sac the epithelium is not everywhere the same. In some places the cells are large and cubical; in other places flattened and less distinct. Often is it impossible to determine whether or not an epithelium is present, for in such places the epithelial cells can not be distinguished from those of the mesenchyma. The mesenchyma surrounding the yolk-sac also varies in thickness. It contains numerous blood-vessels and is covered by the mesothelium of the body-cavity.

The hind-gut, down as far as the cloaca, is composed of but a single layer of cuboidal cells, which is of equal thickness all around. In the cloaca and caudal intestine the epithelium of the side and ventral walls is thickened, and is composed of two to three layers of cells. The entodermal epithelium of the cloacal membrane shows no distinguishing characteristics. It abuts against the ectodermal epithelium, but both layers can be made out distinctly.

## SOMITES.

In all, 24 pairs of somites are present; they extend from the lower end of the hindbrain to a little beyond the point where the allantoic duct passes out from the cloaca. The first somite is found in the region of the eighth and ninth rhombic neuromeres. According to studies on a slightly older human embryo and on young embryos of the pig, sheep, and cat, I have found that this position is normally occupied by the second somite. Watt[48] likewise shows the second somite in this position, the first somite being in relation to the seventh and eighth neuromeres. I have looked repeatedly in my embryo, however, for evidence of another somite in front of the one which I have designated as the first, but have been unable to find any definite indication of such.

As in other young embryos which have been described, the different pairs of somites are found in different stages of development, those nearer the head end always being more advanced than those behind them. In the caudal end of the embryo are found somites in the process of formation; in the head they are already partially broken up. Following Ingalls's[19] plan, I shall begin my description with the somites of the tail and proceed forwards.

The caudal end of the vertebral plates of mesoderm fill up entirely the tail of the embryo around the neural tube, notochord, and tail gut. In the region of the cloacal membrane they appear as solid masses or cords of mesoderm with closely packed cells. At their cephalic ends a small cavity is apparent. Another pair of somites, the twenty-fifth, are partially formed by an incomplete transverse furrow. Numerous mitotic figures are present in the vertebral plates.

The twenty-fourth somite (second lumbar) is in a very early stage of development. It is somewhat cubical in shape and in its center is a distinct cavity, the myocœle. The walls of the somite, which may be described as dorsal, ventral,

medial, and lateral, are all of about equal thickness. They are epithelial in character and contain one to two layers of cells. In the myocœle are found a few scattered stellate cells not unlike mesenchymal cells; later these will enter into the formation of the sclerotome. Mitotic figures are numerous among the cells of the walls and the myocœle, but those of the walls are always at the upper ends of the cells, *i. e.*, the ends bordering on the myocœle. This somite corresponds quite closely with the first coccygeal somite of the Ingalls embryo, except that it probably has fewer cells in its cavity.

The nineteenth somite (ninth thoracic) shows a somewhat more advanced condition. The myocœle in its lower portion is entirely filled with cells. The ventral half of the medial and all of the ventral wall are breaking up. The cells of these walls, together with those on the inside of the myocœle, form the sclerotome. These cells have pushed out slightly toward the chorda dorsalis, forming the notochordal process. The somite corresponds with the sacral somites of Ingalls's embryo.

The fourteenth somite (fourth thoracic) is more distally located from the median plane than the previously described somite. Its ventral and medial walls have both broken down and lost their epithelial character and appear as a mass of mesenchyma between the remainder of the somite laterally, the medullary tube and chorda medially, and the dorsal aorta, cœlomic epithelium, and posterior cardinal vein ventrally. The notochordal and aortic processes of the sclerotome, lying dorsally and laterally to the dorsal aorta respectively, are easily recognized. The lateral wall is somewhat thicker than that of the above-described somite, being composed of apparently two layers of distinct columnar cells. The dorsal edge of this wall is bent first medially, then ventrally, and comes to lie near the median surface of the lateral wall. It is, however, separated from the lateral wall by a cleft-like portion of the myocœle. The dorsal border of this cleft—that is, the groove formed by the rolling over of the medial wall—has been termed by Williams[49] the "upper myotomic groove." At the place where the bent-over portion of the dorsal border of the median wall is in contact with the sclerotome it has left a groove on the medial surface of the somite. This has been called the "lower myotomic groove" by Williams and others. The ventral edge of the lateral wall is also turned in medially but to a lesser degree. The myocœle, which as stated before is cleft-like at its dorsal part, is larger and broader ventrally. Owing to the breaking-down of the medial wall, a wide opening is left in it, the so-called intervertebral cleft. This somite, on the whole, is quite similar to Ingalls's lumbar somites.

The twelfth somite (second thoracic, plate 4, fig. 2), has its sclerotomic cells scattered between the remainder of the somite laterally and the medullary tube and chorda medially. The dorsal edge of its lateral wall has folded over and grown ventrally along the medial surface of this wall, and has united with the turned-up ventral edge except at one place. The lateral wall can now be described as being composed of an outer lamella (cutis plate, dermatome) and an inner lamella (muscular plate or myotome). The intervertebral cleft which lies at the caudal

end of the medial wall is again distinct. This somite is similar in appearance to Kollman's[24] plate 1, figure 1, the myotome of a human embryo of three weeks, but its dermatome contains fewer layers of cells than pictured by Kollman.

In the sixth somite (fourth cervical) the inner lamella is thicker, particularly at its anterior end. The dermatome is also slightly thicker and larger. Its cells, of which there are from one to two layers, are distinctly columnar. Mitotic figures are numerous and again confined to the upper ends of the cells. The myocœle is reduced to a small cleft between the medial and lateral lamellæ. Caudally and ventrally the intervertebral cleft is again seen distinctly. This somite is probably similar to those of the lower thoracic region of Ingalls's embryo.

The fourth somite (second cervical) is not so far developed as the first thoracic as described by Ingalls. It is interesting to note, however, that it is quite similar in structure to the second somite of a 25-segment chick as described by Williams[49]. The dermo-myotome is a flattened quadrilateral body lying just beneath the ectoderm. Its lateral and medial lamellæ are closely approximated, there being no evidence of a myocœle. The breaking-up of the dermatome, as described by many writers, is now beginning, as is indicated by the sending out of a few protoplasmic processes of the outer portion of the dermatome to the covering ectoderm.

The third somite (first cervical) is similar to the fourth, but shows a somewhat more broken-up condition. Its dermotome lies almost in contact with the outer ectoderm and its cells are beginning to send out processes. The cells of the myotome are also beginning to undergo further differentiation, for their spindle-like forms can be made out.

The second and first somites (occipital) are not definitely marked off from each other. The second shows a slightly more advanced condition than the third. The first is small. Its dermo-myotome is distinct, but the outlines of the sclerotome are lost. It is also not definitely separated from the mesenchyma in front of it.

## CHORDA DORSALIS.

Throughout its whole extent, the chorda dorsalis lies just ventral to the medullary tube, the curvatures of which it closely follows. Its anterior end, which begins opposite the point at which the remnants of the oral membrane are attached to the roof of the mouth, is flattened dorso-ventrally and makes a slight bend to the right. Caudal to this flattened portion, the chorda assumes in general a cylindrical shape, although in some places it is flattened either dorso-ventrally or laterally, while in other places it is triangular in cross-section. It terminates caudally in the tail by joining the undifferentiated cells of the primitive-streak region (text-fig. 9).

An examination of the chorda dorsalis shows that it is not everywhere of uniform size, but that it is alternately expanded and constricted. In order to determine whether the expanded portions are arranged in any way with reference to the body segments, a wax reconstruction of a portion of the chorda was made; but owing to its irregular shape it is difficult to determine from the reconstruction

which portions are actually expanded and which constricted. A segment which appears expanded in side view may appear constricted from the front or back. Relative measurements of cross-sectional (oblique) areas of the chorda dorsalis between the second and thirteenth segments seem to indicate that the expanded portions lie within the body segments, but the evidence for this is not altogether convincing.

That segmental flexures of the chorda dorsalis exist is indicated by the fact that for long distances, including extents of a number of segments, the chorda lies approximately equidistant from the medullary tube. The ventral surface of the medullary tube, as shown in plate 2, figure 1, presents dorsal and ventral curvatures; consequently the chorda must also follow these curvatures. This would make the ventral curvatures of the chorda segmental. Minot[37] has described, under the term "segmental flexures of the notochord," a series of dorsal and ventral curves which he found to be constant in a number of mammals, man included; these, he states, are so placed that their dorsal curvatures are segmentally arranged, but he states further that in young pig embryos the ventral curves are segmental and that a shifting of the flexures takes place in embryos of about 12 mm. The flexures which are apparently present in my embryo, therefore, accord with those which Minot describes for pig embryos younger than 12 mm.

The chorda is fused with the entoderm of the digestive tube in one region only, namely, in the posterior part of the pharynx at about the level of the third to fourth body segments. Here it is attached in two places, each extending through but a few sections and the two fusions separated from one another by but three sections. The more anterior fusion is shown in text-figure 7. It is of interest to note that Gage[12] and Thompson[44] both found a fusion of the chorda to the entoderm in the same location and that Watt[48] shows the chordæ in the twin embryos he describes to be least developed in this region. Watt believes, therefore, that that section of the chorda opposite the posterior part of the pharynx is the last in point of time to develop, and that embryos showing a fusion of chorda and entoderm in this region are evidence in favor of this view.

Throughout its whole course the chorda dorsalis is invested on either side with mesenchyma. Dorsally and ventrally, however, this investment is not complete. Ventrally it is incomplete not only at the points of fusion with the entoderm, but both anteriorly and posteriorly to them. Anteriorly it lies almost in contact with the roof of the greater part of the pharynx (text-fig. 6). Posteriorly it soon becomes separated from the pharyngeal wall by intervening mesenchyma. From the seventh body segment caudally the chorda lies almost in contact dorsally with the medullary tube, there being from this point caudally no mesenchyma on the dorsum of the chorda. Elsewhere the chorda is completely surrounded by mesenchyma. The younger chordæ described by Watt showed mesenchyma ventrally in one small region only, namely, at the level of the eighth segment, while dorsally mesenchyma passed between the chorda and medullary tube in two places—at the anterior end and again opposite the first body segment. The rapidity with which the notochodral processes of the sclerotomes separate

the chorda from the medullary tube dorsally and the digestive tube ventrally can be noted by a comparison of the specimens. Its dorsal separation seems to begin anteriorly and to proceed caudalward. Ventrally separation apparently begins in the middle region of the body and progresses both anteriorly and posteriorly. Another point of ventral separation begins anteriorly and progresses caudally, the last portion to become separated undoubtedly being that which is fused to the pharynx.

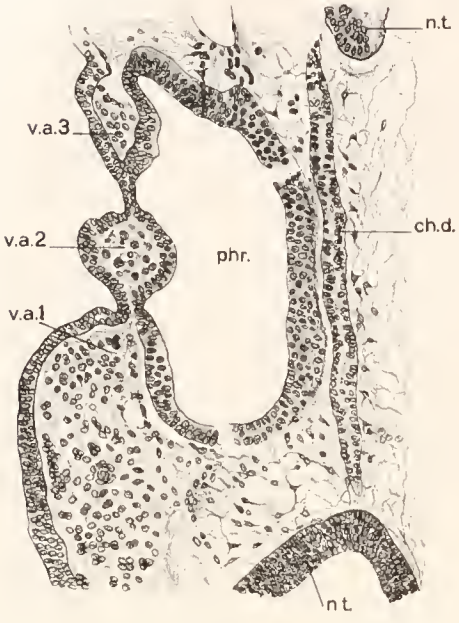


FIG. 6.—Portion of section 42. ×125 diameters.

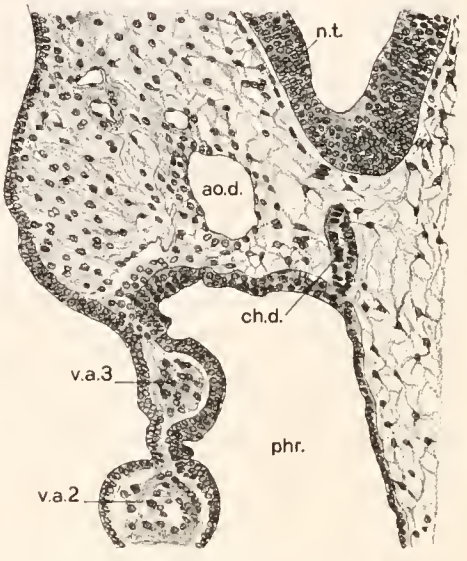


FIG. 7.—Portion of section 46. ×125 diameters.

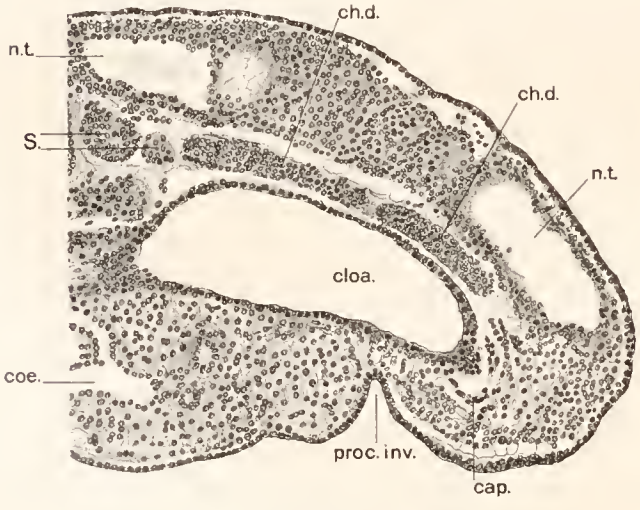


FIG. 8.—Portion of section 218. ×125 diameters.

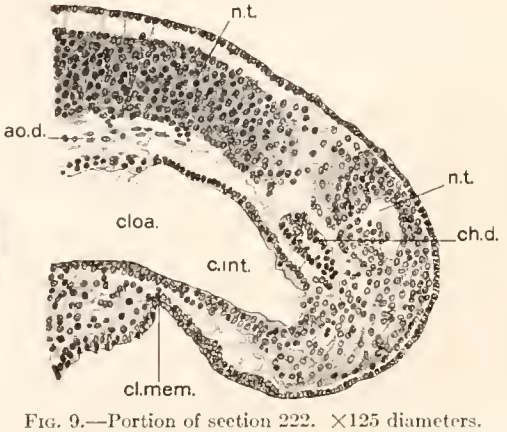


FIG. 9.—Portion of section 222. ×125 diameters.

Watt describes what he considers to be neurenteric canals in his twin embryos. They are situated just dorsal to the cloaca and consist of a connecting rod of cells joining the chorda with the medullary tube dorsally and with the entoderm ventrally. This region in my specimen is cut through sagittally, but as seen in text-figures 8 and 9, no evidence of such a cord of cells is present.

The chorda dorsalis consists of polygonal and wedge-shaped cells, with large rounded nuclei and considerable granular cytoplasm. In places the cells are arranged radially about the center of the chord, while in other regions this arrangement is less distinct. Mitotic figures are numerous among the cells.

In the center of the chorda a fine lumen can be made out in certain sections (plate 4, fig. 3). This lumen is not continuous throughout, but is present in numerous places, each extending through but a few sections. In size it varies from 2 to 4 microns. That this discontinuous lumen of the chorda is normal in certain young stages of the human embryo seems to be well established, since it has been found by His[18] in his embryo L1 of 2.4 mm., by Eternod[5] in three embryos of 1.3 mm., 2.11 mm., the third somewhat larger, and by Watt[48] in twin embryos of 17-19 paired somites.

An ill-defined cuticular membrane surrounding the chorda dorsalis, such as has been described by Van den Broeck[47], is present, except where the chorda is fused to the entoderm of the pharynx.

## NEPHRIC SYSTEM.

In describing the Robert Meyer embryo of 23 somites (Thompson's embryo) Felix[10] states:

> "The pronephros is almost completely developed, so far at least as one may speak of its completion. It consists of a number of tubules and the primary excretory duct. There are in all seven tubules present, the most anterior of which is not united with the succeeding tubule . . . . The tubules 2–5 have fused so as to form a collecting duct. The tubules 5, 6, and 7 are not yet united, but their union is imminent."

In my specimen I likewise find a series of tubules representing the nephric system. Although conditions on both sides of the embryo are not identical, for the most part they are quite similar. The following description is based on their arrangement of the left side and the terminology used is the same as employed by Felix.

On the seven pronephric tubules which are present, the first two are rudimentary (plate 4, fig. 4). The first is represented by two or three small clusters of cells at the level of the ninth body-segment. It is indistinct and indefinite and its identity is determinable only by the position which the clusters of cells occupy and by the fact that the cells are more or less isolated from the mesenchyma.

The second tubule is larger and more distinct. It is composed of a single cluster of cells which form a spherical mass. Its cells are isolated from the mesenchymal cells by a clear space. It also lies in the seventh body-segment. The remaining pronephric tubules are elongated gland-like tubes of epithelium, rather bulbous at their cephalic extremities and tapering caudally. Each in its course crosses the body-wall obliquely in a ventro-dorsal direction; thus in its upper part each lies close to the mesodermal lining of the cœlomic cavity, while below it comes in close relation with the ectoderm (plate 4, fig. 4). According to the description of Felix, each of these tubules represents two different portions of the

pronephros. The bulbous portion is the inner pronephric chamber. It is connected to the mesothelium of the cœlomic cavity by a slender strand of cells, the nephrostome, which joins it ventrally. The caudal portion of the tubule Felix terms the principal collecting tubule.

The third pronephric tubule is the longest. It is rounded in cross-section and more regular in form than any of the succeeding tubules. The inner pronephric chamber is small cephalically, but is not connected to the mesodermal lining of the body-cavity by means of a nephrostome. A pit on the mesodermal lining of the body-cavity opposite the tubule, however, probably represents a broken-down nephrostome. The principal collecting tubule extends caudally close to the ectoderm. It terminates in relation with the principal collecting tubule of the fourth pronephric tubule. Whether an actual fusion exists between the two collecting tubules or whether they merely touch, I am unable to determine. The third pronephric tubule possesses a distinct lumen, which extends throughout most of its length. It lies in the tenth body segment, its collecting tubule extending into the eleventh segment.

The fourth tubule is almost the same shape and size as the third and is less regular in form. Again a connecting nephrostome is lacking, although its position is marked on the free surface of the mesothelium by a depression. The principal collecting tubule ends in connection with that of the fifth tubule. The joined distal ends of the collecting tubules give rise to the primary excretory duct. The fourth tubule possesses a discontinuous lumen divided into two portions.

The fifth (plate 4, fig. 2), sixth, and seventh tubules differ from the third and fourth in possessing connecting nephrostomes. Each nephrostome begins as a funnel-shaped opening on the cœlomic wall. In no case, however, can a lumen be traced into the tubule. Each nephrostome is joined to its inner pronephric chamber by a strand of cells. Of these, that of the fifth tubule is extremely long and band-like. The inner pronephric chambers are definite swellings situated near the middle of the tubule. That of the seventh is the largest. The principal collecting tubules are joined by the preceding ones as shown in plate 4, figure 4. Lumens are present only in the inner pronephric chambers. The fifth and sixth pronephric tubules are situated in the twelfth body-segment, while the seventh is found in the thirteenth.

Histologically the tubules in all parts are made up of polygonal or columnar cells, with rounded or elongated nuclei. Where a lumen is present the cells are arranged radially about it, but in other places there seems to be no definite arrangement.

Beyond the seventh pronephric tubule and apparently continuous with it is a large rounded cord of cells which extends through the remainder of the embryo. This cord possesses a number of irregular swellings, the mesonephric vesicles. These vary in size and shape and are not so definitely marked off from one another as shown by Felix[10] and Watt[48]; 16 to 18 may be counted (plate 4, fig. 4); they possess nephrostomes connecting them with the cœlomic mesothelium similar to those of the pronephric tubules.

The primary excretory duct occupies a position just dorsal to the nephrogenic cord. In its course caudally it lies close to the ectoderm. Felix was unable to determine whether it developed from the ectoderm or whether it arose independently in the mesoderm, but he doubts that the ectoderm has any participation in its development. Watt[48] also was unable to determine definitely its mode of formation. The caudal end of the primary excretory duct, the point at which its formation is supposed to be taking place, is in my embryo cut sagittally. It is, however, indefinite. Just before its termination the duct is seen lying close to the ectoderm in the mesenchyma. The mesenchyma possesses several mitotic figures in the region in which it terminates. While I am inclined to favor Felix's view of a mesenchymal origin, the evidence found is not convincing.

## VASCULAR SYSTEM.

### HEART.

The heart lies in that portion of the body-cavity which is bounded by the pharynx above, the fore-gut behind, the anterior body-wall in front, and the transverse septum below. It is still a simple tube and viewed from in front is roughly the shape of the letter U. It is placed so that the loop of the U is directed toward and lies in the right side of the pericardial cavity. The limbs of the U are turned to the left, but upon reaching the body-wall of the left side turn dorsalward at an angle of almost 90 degrees. The upper limb then bends sharply cephalad, joins immediately the pericardial wall, and passes into the ventral aorta. The lower limb bends medially and joins the pericardial wall as it passes into the sinus venosus. The ends of the heart-tube are therefore fixed to the body-wall, but the remainder of the heart lies free within the cavity.

An examination of the heart-tube shows that it is not everywhere of the same caliber, but presents certain expanded portions separated by more or less definite constrictions. Beginning with the venous end of the heart, the sinus venosus passes into the atrium with but a slight constriction. Figure 2, plate 1, shows that portion of the heart which I regard as the atrium. As seen from behind (plate 3, fig. 1), it presents a V-shaped bend, the apex of which is pointed toward the left, the limbs lying in a horizontal plane. On its upper surface is a distinct irregular projection, as to the significance of which I am in doubt.

The atrial portion of the heart passes into the ventricular portion with only a slight constriction, the atrio-ventricular canal. The ventricle is much enlarged, having a transverse diameter which is greater than that of any other portion of the heart. It fills up the entire lower right-hand portion of the pericardial cavity. Its cephalic end is bounded by a shallow constriction, in front of which is the bulbus cordis.

The bulbus cordis is large where it is attached to the ventricle, but gradually becomes narrower towards its cephalic end. It is directed downwards and towards the left. As described by Watt and others, it reaches farther cephalad than any other portion of the heart. It becomes continuous with the truncus

arteriosus, a short, narrow portion of the heart-tube which is directed dorsally and towards the left. The truncus arteriosus bends sharply upward and joins the ventral aorta.

The cavity of the heart approximately follows the center of the heart-tube. Like the outside of the heart, it also is not of uniform diameter, but presents swellings and constrictions, as shown in plate 3, figure 2. The sinus venosus passes to the left as an irregular tube. It is marked off from the atrium by a slight constriction. At the apex of the atrial bend the cavity enlarges considerably. On the superior surface of this enlargement is a crescentic projection, the concavity of which is directed medially. This extends into the above-described projection of the atrium as seen from the surface.

Between the atrium and ventricle is a long, narrow portion of heart-cavity, the atrio-ventricular canal. The difference in size between this narrow portion and that of the adjacent swellings is greater than that between the same portions of the heart as seen from its surface. The cavities of the ventricle and bulbus cordis are again enlarged and separated from one another by a rather long constriction portion. This is again much narrower proportionally than the same constriction seen from the surface of the heart. In cross-section the cavities of the ventricle and bulb, particularly the former, are triangular. In the truncus arteriosus the cavity is again small and irregular. Cephalad it gradually widens out as it becomes the ventral aorta.

The endothelium of the heart is everywhere a syncytium of one layer of cells. Its nuclei are oval in shape and coarsely granular, while the cytoplasm stands out sharply in contrast to the coagulum without. In the bulb and ventricle, at the angles of the cavity as seen in cross-section, the endothelium sends out plate-like processes of cells. These give to the various portions of the endothelial heart a stellate appearance. In some instances these processes extend to the myocardial layer and apparently fuse with it. One such process is shown in plate 4, figure 1. It passes off from the endothelial tube of the heart as a solid cord of cells. It extends entirely across the space between the endothelial and myocardial layers and terminates in a bulbous expansion in contact with the myocardium. In the expanded end is seen a distinct mitotic figure. The significance of processes of this kind can not be doubted. They represent the earliest beginnings of the so-called "heart sinusoids," which in embryos a little older are numerous. By their growth and anastomoses they give rise to the trabeculæ of the heart.

In the atrium, with the exception of the above-described atrial projection, the endothelium lies in contact with the myocardium, there being no intervening space such as is found in the remainder of the heart-tube. This relation of the endothelium to the myocardium in the atrium has been noted by His[18]. The same condition has been described by Mall[33], who states:

> "This arrangement is so pronounced in the early heart that it affords a way by which we may determine with precision the exact portion of the heart tube from which the atrium arises."

No mention, however, is made by Mall, or by Watt[48], who describes a similar arrangement, of that portion of the atrium which projects upward and in which the endothelium is not closely applied to the myocardium.

The outer layer of the heart-tube, the so-called myo-epicardial layer, is composed of several layers of cells, the thickness varying in different portions of the heart. As Tandler[44] has shown in an embryo of 3.5 mm. (embryo Hal, Institute of Anatomy, Vienna), these cells form a distinct syncytium. The myo-epicardium is directly continuous at the venous and aortic ends of the heart with the transverse septum and lining of the body-cavity, respectively, and it is by means of these attachments that the heart is held in place.

In that portion of the atrium where the myo-epicardium lies in contact with the endothelium, its cells are closely applied to one another. Nuclei are crowded and intercellular spaces are small. The myo-epicardium of the atrio-ventricular canal is somewhat thicker than that of the atrium, and its cells are more widely separated. Numerous small protoplasmic processes of the cells form a network, the meshes of which are filled with delicate fibrils. In the ventricle and bulbus cordis the myo-epicardium is still thicker, but in the truncus arteriosus it again becomes thin.

In describing the 3.5 mm. embryo, Tandler[44] says:

> "The myo-epicardial mantle differentiates to the extent that in the region of the ventricular loop and in the bulbus its superficial layer is formed by a continuous row of cells, the epicardium, while on the atrium and sinus, so far as the latter has a free surface, no such differentiation can be said to exist."

In my embryo I find that the outside layer of cells of the myo-epicardium have not yet become flattened and detached from the underlying cells, as shown by Tandler in his figure 377, but over the entire surface of the heart they are arranged in a distinct layer (plate 4, fig. 1). As seen in cross-section, they are in places cubical and closely packed, while in other places they are more rounded and farther spread apart. I am also unable to find any differentiation of the myocardium proper into an inner spongy portion and an outer cortical portion, as Tandler describes for the 3.5 mm. specimen.

The broad space which exists between the myo-epicardial and endothelial layers, according to Tandler, is filled during life with serous fluid, since it is occupied in section by a clot-like fibrous mass which is entirely destitute of cells and stains feebly with the hemotoxylin. In my specimen I find a similar clot-like mass. The small, delicate fibrils form an anastomosing plexus, the meshes of which are empty. For the most part these fibrils extend radially from the endothelium to the myocardium, to both of which they gain attachment. They radiate out particularly from the plate-like processes of the endothelium, making it impossible to determine in every case where the endothelium leaves off and where the fibrin begins. It seems probable that the delicate fibrils found in the myocardium are due to a similar coagulation of serous fluid.

Mitoses within the tissue of the heart are few. Occasionally one may be observed in the endothelium, particularly in the cells of its plate-like processes.

In the myo-epicardium, with the exception of that of the sinus venosus, they are extremely rare. In the sinus venosus, and particularly in those portions of the mesothelium of the body-cavity and transverse septum which are directly continuous with the myo-epicardium, mitotic figures are comparatively numerous. This finding would seem to indicate that the multiplication of cells of the myo-epicardium of the heart takes place at this stage principally in the region of the sinus venosus.

## VEINS.

### VENA CARDINALIS ANTERIOR.

The vena cardinalis anterior (plate 5, fig. 1) draws its blood principally from the region of the brain. In Ingalls's embryo this vein begins at the junction of two veins which course caudally from the region of the prosencephalon. Ingalls, basing his interpretations upon the work of Mall[31], regards the dorsal of these as the source of the future sinus sagittalis superior; the ventral one, the vena ophthalmicus. In my specimen the region drained by the two above-mentioned veins is occupied by a venous plexus. One tributary, which extends upward from the region of the optic vesicle, may already be identified with reasonable certainty as the ophthalmic vein. The much more extensive plexus above probably gives rise to the embryonic superior sagittal sinus. The tributaries of the above-described plexus come together medial to and behind the trigeminal ganglion, where they form an enlarged venous sinus, between the trigeminal ganglion in front and the acustico-facial ganglion behind. From this the anterior cardinal vein passes caudally by two main channels, the dorsal of which is situated above the origin of the acustico-facial ganglion, while the ventral one is placed medial to it. Passing the interspace between the acustico-facial ganglion and otocyst, these branches unite again medially to the otocyst, forming another enlarged portion of the vein. Caudal to the otocyst it divides into three smaller veins, which soon come together again. Opposite the first somite the anterior cardinal vein becomes very small in diameter, but gradually becomes larger when traced still farther caudally. From the second segment to its termination, the vena cardinalis anterior is represented by two small veins which are closely related to one another. In several places, however, they unite to form a single vessel. At the level of the fifth body-segment the vena cardinalis anterior enters the vena cardinalis communis (duct of Cuvier).

In its course the vena cardinalis anterior receives tributaries on both its dorsal and ventral walls. On the dorsal, the first is found in the region of the trigeminal ganglion and proceeds from the direction of the mesencephalon to reach the anterior cardinal vein at the posterior border of that ganglion. The second lies just in front of the otocyst close to the rhombencephalon. The position which it occupies (just in front of the otocyst) indicates that it is the same vein which Mall[31] describes as the vena cerebri media. Behind the otocyst is a small stump of a vessel which could not be traced far dorsally. Owing to its position, just behind the otocyst, it becomes evident that this vein must be identical with the one occupying a similar position in Ingalls's embryo and which Evans[8] has inter-

preted to be the vena cerebri posterior. Just caudal to this vein is a longer vein, which, arising in the region of the first segment and extending anteriorly and ventrally, joins the anterior cardinal vein at the point where the latter is broken up into two portions. I am in doubt concerning its identity. Several small dorsal tributaries of varying size are received throughout the remainder of the vena cardinalis anterior. They include the lateral loops of the second, third, and fourth dorsal segmental arteries, which are described below.

The ventral tributaries are more numerous than the dorsal. They may be described as belonging to the different visceral arches. One arises in the mandibular arch close to the mouth, passes dorsalwards, and unites with a network of small veins. The blood from this tributary may reach the anterior cardinal vein, either in the region of the trigeminal or of the acustico-facial ganglion.

The venous tributaries of the second arch do not arise as far down as those of the mandibular arch. They form a plexus which lies in close relation to the facial nerve. One tributary passes medially to the ganglion, while the others are laterally situated. There is thus established about the acustico-facial ganglion a venous ring. The plexus anastomoses with that of the first arch.

In the upper part of the third arch are found three small tributaries, which unite and reach the anterior cardinal vein as a single vessel. No others are found in this visceral arch. Farther caudally, opposite the second and third somites, several small veins unite with the anterior cardinal. They probably represent similar tributaries from the fourth arch.

At the point at which the anterior cardinal veins empty into the common cardinal there is received, on the ventral side, a long, slender vein (vena linguofacialis). The smallest tributaries of this vein may be traced as far as the third visceral arch. Uniting, these tributaries form the vein which proceeds caudally and dorsally. In its course it passes in the antero-lateral body-wall over the heart. A similar vein has been described by Ingalls[19], as follows:

"Am Anfang des Ductus Cuvieri münden in ihn auf jeder Seite je ein von der vorderen Bauchwand kommendes Gefäss. Auf der rechten Seite ist dies besonders gross, es lässt sich ventralwärts bis in die Nähe des Ursprungs der ersten Aortenbogen verfolgen and weiter Kaudalwärts bis dahin, wo die ersten Kiemenbogen mit der vorderen Körperwand verschmolzen sind, um sich schliesslich in dem ersten Bogen zu verlieren."

The same vein had been found by Salzer[40] in embryos of the guinea-pig and later by Grosser[16] in bat embryos. Lewis[26] describes it in the pig embryo under the term "transverse vein," and later[27] discussed its origin and fate in rabbit and human embryos. Apparently the first reference to this vein, which is now recognized as of constant occurrence and fundamental morphological importance, was made by Phisalix, as Dr. Lewis has pointed out to me. Phisalix[39], in 1888, showed it clearly in a figure of a 10 mm. human embryo and described it as follows:

"Entre la veine jugulaire et la veine cardinale se trouve une vaste poche dont le sang s'écoule par les canaux de Cuvier . . . . En avant et au-dessus, chacune de ces poches reçoit des veinules qui accompagnent le nerf hypoglosse et qui viennent de la base des arcs branchiaux."

In injections of the veins of pig embryos, Smith[41] shows definite anastomoses between the linguo-facial vein and the venous plexuses of the visceral arches. The anastomosis between this vein and the plexus of the first visceral arch is apparently already formed in Ingalls's 4.9 mm. human embryo. In my embryo I have been unable to find an anastomosis, but the direction which the vein takes (plate 5, fig. 1) seems to indicate the possibility that these two sets of tributaries might soon unite.

### VENA CARDINALIS POSTERIOR.

The blood from the tail and lower half of the embryo is carried to the vena cardinalis communis by means of the vena cardinalis posterior. This arises in the tail of the embryo in the region of the unsegmented mesodermal plates as a slender irregular vessel. In its course to the bend in the back it passes along the ventro-lateral border of the somites, with which it lies in contact. In this part of its course, owing to the plane of sectioning, it is difficult to trace its capillary connections, but in places there is evidence that connections similar to those above the nineteenth somite exist below it.

For the most part that portion of the posterior cardinal vein from the bend in the back to the common cardinal stands out quite sharply. In general it courses along the ventro-lateral border of the somites, lying between them and the pronephros or mesonephros. Opposite the somites of the seventh to eleventh segments the posterior cardinal vein becomes very difficult to follow. It is represented by a slender and apparently solid cord of endothelial cells. In the intersegmental spaces the vessel becomes larger and usually contains a number of blood-cells (plate 5, fig. 1). Opposite the ninth somite (and again the tenth) the existence of the vessel becomes doubtful, owing to the similarity of endothelial to mesenchymal cells. In the corresponding region on the right the continuity of the posterior cardinal vein is even more doubtful. Whether this is due to a closing-up of a once continuous posterior cardinal vein or whether it represents an incompletely developed vein is impossible to determine from the sections. According to Evans[8], the posterior cardinal veins first appear in human embryos possessing from 15 to 23 somites. He states:

> "It is probable that lateral loops of the dorsal segmental arteries are instrumental in the formation of these veins, as in the case of the anterior cardinals. This method of formation of the posterior cardinal veins appears fundamental. Raffaele (1892) and Hoffman (1893) describe it for selachian embryos and Grafe (1905) and the writer have indicated it in the case of the chick."

It is probable, therefore, that in my embryo the above-mentioned portions of the posterior cardinal veins are still in the formative stage, particularly since the intersegmental portions of the vein, to which are joined the lateral loops of the segmental arteries, are more definitely marked than the segmental portions.

From the interspace between the seventeenth and eighteenth segments to its termination, the posterior cardinal vein receives its blood principally from the small lateral loops of the dorsal segmental arteries, as has been described by Evans[8].

In addition to these tributaries, connections can be made out in several places between the posterior cardinal vein and the lateral branches of the aorta, but these connections are not so distinct as the branches from the dorsal segmental arteries. In the regions of the pronephros and mesonephric vesicles small tributaries are found arranged in pairs, one lying on either side of these organs; both pass dorsally to join the posterior cardinal vein. The lateral tributaries pass, in the region of the pronephros, between the inner pronephric chambers of one pronephric tubule and the principal collecting tubule of the preceding one. Farther down, in the region of the mesonephros, they pass between the mesonephric vesicles and the primary excretory duct.

Evans[8] shows similar vessels in a reconstruction of the 23-somite embryo of Robert Meyer. He also indicates a longitudinal vein which connects the peripheral ends of the medial tributaries and another similarly connecting the lateral tributaries; these he terms the medial and lateral subcardinal veins, respectively. I have been unable to make out continuous longitudinal connections in my specimen, but indications of them are apparent on a few of the medial vessels. I find, however, connections between the medial tributary and the lateral segmental arteries, such as Grafe[13] has shown in the chick and Evans[8] notes in the Robert Meyer specimen. It is interesting to note that the two above-described tributaries of the posterior cardinal vein are not located with reference to the segments, but (as Evans and others have described for the lateral segmental arteries) they correspond quite closely in number and position with the pronephric tubules, where the latter are present. Below the pronephric tubules they are arranged with reference to the mesonephric vesicles.

### VENA CARDINALIS COMMUNIS.

The common cardinal vein (duct of Cuvier) is a short, flattened vessel which lies within the sixth body segment. It receives both the anterior and posterior cardinal veins. It is directed caudally in the lateral body-wall and, breaking up into three portions, joins the vena umbilicalis at the point where this vein enters the transverse septum (plate 5, fig. 1, and plate 6, fig. 1).

### VENÆ UMBILICALES.

The umbilical veins begin at the distal end of the body-stalk by the union of several large veins which drain the chorion and its villi. At first they form a single vessel, which soon, however, breaks up into a plexiform arrangement of large veins (plate 5, fig. 1). These reunite to form a single large vein, which again divides to form two smaller vessels, the right and left umbilical veins. Immediately upon separating they pass to the outer border of the umbilical stalk, one on either side, and enter the body-wall.

In the beginning of its course the right vein is very small. Soon, however, it increases in size and in the remainder of its course it is similar to and about as large as the left umbilical vein, which is quite uniform throughout. Each umbilical vein, throughout its entire course from the body-stalk to the septum transversum, lies within the body-wall, situated in this at about the junction of its

middle and distal thirds. Each receives from the body-wall tributaries coming from both dorsal and ventral directions. The tributaries which reach the umbilical vein on its ventral wall arise for the most part within villus-like processes of the body-wall. On the left side, one of these (found at the level of the tenth and eleventh body-segments) is even larger in cross-section than the main stem of the vein itself and forms a venous sinus in the villus (plate 5, fig. 1.) It is drained by a relatively small vessel. Above this are a number of tributaries which drain a longitudinal vessel situated in line with the venous sinus below. Concerning the significance of these vessels I am in doubt, but believe them to be either the remnants of the plexus from which the umbilical vein has developed (Evans[6]) or the beginning of the anterior body-wall plexus (Smith[41]).

Opposite the seventh somite the left umbilical vein receives a branch from the vitelline vein, which lies ventral and caudal to the main junction of these vessels. Approximately at the level of the interspace between the sixth and seventh body-segments, the left umbilical vein receives from above the vena cardinalis communis, entering by three distinct tributaries (plate 5, fig. 1), as described above. Turning sharply medially into the septum transversum, it unites with the vitelline vein (plate 6, fig. 1), and with it forms the left vitello-umbilical trunk. This trunk, which is represented by one main vessel and two smaller ones, passes medial to join the sinus venosus.

The right umbilical vein likewise receives tributaries from the body-wall all along its course and at its most cephalic point receives the right common cardinal vein. It is united to only a portion of the vitelline vein and at but one point. The vitello-umbilical trunk is represented on this side by a network of smaller veins which connect it with the sinus venosus.

### VENÆ VITELLINÆ.

The vitelline veins arise on the surface of the yolk-sac from the yolk-sac plexus. Two principal veins, the right and left, are formed on the yolk-stalk by the convergence of numerous tributaries. These course cephalad, one on either side, and enter the septum transversum (plate 6, fig. 1). They pass dorsally along the sides of the hepatic diverticulum, lying quite close to it and the wall of the fore-gut. Several small tributaries proceeding from the mesenchyma surrounding the fore-gut are received by them from above. Each breaks up into several branches, which form a plexus within the transverse septum. A minute commissural branch connecting the veins of both sides is found in the notch between the hepatic diverticulum and the fore-gut wall. The two connections of the left vitelline vein with the left umbilical vein have already been described. All the blood carried by the left vein, except that which may cross over to the opposite side in the small commissural branch, joins that of the umbilical vein before reaching the sinus venosus. Near its termination the right vitelline vein breaks up to form a plexus, the branches of which diverge. Some of these pass directly into the sinus venosus, while others join the umbilical vein to form a plexus representing the right vitello-umbilical trunk. Only a part of the blood which is carried by the right vitelline vein, therefore, passes through the right vitello-umbilical trunk.

Over the yolk-stalk the tributaries of the vitelline veins form a network of small vessels. These reach dorsally as far as the middle of what might be outlined as the gut. Extending along the gut-wall, therefore, are a number of small longitudinal coursing veins (plate 5, fig. 1). They can be traced caudally along the hind-gut for some little distance below the hind-gut portal. The significance of this plexus I have not been able to determine definitely, but I judge that it will ultimately give rise to the inferior mesenteric vein.

#### SINUS VENOSUS.

The sinus venosus is situated within the substance of the septum transversum, ventral to the gastric region of the fore-gut and cephalad to the tip of the hepatic diverticulum. It is a broad, irregular vessel (text-fig. 4 and plate 5, fig. 1), much flattened dorso-ventrally. On its left border it receives three vessels which represent the left vitello-umbilical trunk. On its right it receives three small branches from the right vitello-umbilical trunk and as many more directly from the right vitelline vein. The sinus venosus curves ventrally and to the left and, becoming a more rounded vessel, passes out of the transverse septum into the atrium of the heart.

### ARTERIES.

#### AORTA VENTRALIS.

The ventral aorta (plate 2, fig. 3, and plate 3, figs. 3 and 4), the direct continuation of the truncus arteriosus, is an unpaired median vessel. It is situated just ventral to the thyroid diverticulum, with which it lies in contact. It at once breaks up into the aortic arches.

#### AORTIC ARCHES.

Three pairs of aortic arches are present (plate 5, fig. 2). Of these the first is by far the largest. Each vessel begins at the anterior extremity of the ventral aorta and extends anteriorly and dorsalward in the first visceral arch, just cephalad to the first pharyngeal pouch. Reaching the upper extremity of the arch, it joins with the dorsal aorta. Both first arches are distinctly patent throughout.

The second and third aortic arches are smaller and less distinct vessels, lying within the second and third arches respectively. The vessels of these arches vary in size and distinctness in different regions. In certain places they become so small that a lumen is no longer discernible, and it becomes impossible to determine whether the vessels are continuous or not. It is very probable, however, that connections do exist at these places, but, owing to the great similarity between the endothelial cells and those of the surrounding mesenchyma, the former can not be traced through with any degree of certainty. On the left side the second arch shows two such doubtful interruptions, one at the point where it leaves the ventral aorta, the other where it joins the dorsal aorta. Between the two breaks a distinct vessel is present. The third arch on the left side arises from the dorsal aorta as a small plexus and extends as a distinct vessel halfway down to the ventral aorta. Here it disappears for several sections, but soon reappears as an apparently solid string of endothelial cells. This cord when traced downward is found to connect with the ventral aorta. On the right side the second and third aortic arches like-

wise can not be traced from dorsal to ventral aorta. The apparently absent portions of both of these arches are near the ventral aorta.

As shown in plate 5, figure 2, there is on the left side a small arterial twig which branches off from the dorsal aorta just behind the third arch. The significance of this is uncertain, but from its position it seems quite probable that it may be the beginning of a fourth aortic arch.

Whether the second and third arches are not yet completely developed or whether their lumens have become secondarily occluded I am unable to determine. It would seem, however, that the former is the more probable, even though in the somewhat younger embryos of Watt and Van den Broeck,[47] and in Thompson's embryo as described by Felix, the second arch is complete. The beginning third arch (and the probable beginning fourth) indicates that in this respect my specimen is older than any of the above-mentioned embryos. The incomplete second arch may be regarded as having been slightly retarded in its development.

### AORTÆ DORSALES.

The dorsal aortæ are two large vessels which extend from about the level of the anterior end of the chorda dorsalis to within a very short distance from the tip of the tail. Between the eighth and nineteenth segments the dorsal aortæ are fused together in the mid-line, forming a single median vessel; elsewhere two distinct vessels are apparent. Where two vessels are present they lie one on either side of and slightly ventral to the notochord; where single it lies directly ventral to the notochord. Throughout their entire courses, whether paired or single, the dorsal aortæ lie just dorsal to the digestive tube.

The dorsal aortæ, when traced from their anterior to their posterior extremities, continually change in shape and size. The median dorsal aorta is smallest in cross-sectional area at about the level of the eleventh segment and largest at the level of the fourteenth. The vascular bed (cross-sectional area) at this level is even larger than that of the paired dorsal aortæ combined. The endothelium of the dorsal aortæ is distinct throughout. In the region of the fourteenth segment it forms an incomplete septum, undoubtedly the remains of the originally fused medial walls of the paired vessels, which have not as yet disappeared.

### BRANCHES OF THE DORSAL AORTÆ.

*Anterior Branches.*—At the point where the dorsal aorta and the first aortic arch join, two small arteries (plate 5, fig. 2) are given off from the dorsal wall of the dorsal aorta. The one situated more caudally is the smaller of the two. It extends dorsally and medially and terminates in the region of the posterior end of the prosencephalon, a short distance behind the anterior end of the notochord. I am unable from the specimen or from other descriptions to identify this vessel with any degree of certainty, but presume that it gives rise to one of the cerebral arteries. The anterior branch is larger. It extends medially and anteriorly and comes to lie close to the side-wall of the prosencephalon. Here it divides into two branches, one of which extends forward on the ventral wall of the prosencephalon, while the other passes medially along its ventral wall. The latter

branch terminates near the mid-line of the embryo not far from its fellow of the opposite side. In addition, the anterior branch gives off along its course two or three small arterial twigs. These pass dorsally along the wall of the brain, and probably represent the beginning of the cerebral arteries.

*Dorsal Segmental Arteries.*—The dorsal segmental arteries are represented by 24 paired vessels. Although for the most part they are similar in position and distribution, they vary greatly in size and in the distinctness with which they can be traced. The following description refers only to those of the left side.

In his account of these vessels of the Robert Meyer embryo No. 300, Evans states[8]:

> "At this stage the dorsal segmental vessels form in the tissue of the intersomitic clefts large, well-marked vascular arches or loops, one limb of which is against the neural tube, while the other joins the cardinal vein."

Evans[7] had previously shown similar loops in chick embryos.

In my embryo I find that loops are present or indicated in case of all of the upper 18 dorsal segmental arteries except the first. Two such typical vessels are shown in plate 6, figure 2. Each dorsal segmental artery extends dorsally and laterally from the aorta. When it reaches a point about halfway up the medullary tube it branches, sending a short branch mediallly and a longer one laterally. The lateral branch extends through the intersomitic cleft and reaches the posterior cardinal vein as described by Evans. The medial one, I find, soon divides into two distinct smaller branches, a dorsal and a ventral. These extend along the wall of the medullary tube and tend, with their fellows of the opposite side, to encircle it.

The first dorsal segmental artery has a relatively extensive origin from the dorsal aorta. It extends dorsalward between the first and second somites, where it breaks up into several branches. One of these extends for a short distance anteriorly, while others extend caudally toward the second dorsal segmental. A connection between the two, however, is apparently not yet formed. I am also unable to trace a connection between this artery and the anterior cardinal vein. In Ingalls's specimen of 4.9 mm. the first dorsal segmental artery (known also as the hypoglossus artery) likewise sends branches both anteriorly and posteriorly. The posterior branch, however, has joined the second dorsal segmental artery, while the anterior is much longer and is easily recognized as the arteria vertebralis. In my specimen, therefore, one sees the very beginning of the formation of the vertebral artery.

The second and third dorsal segmental arteries show only lateral and dorsal branches, while for the fourth only pieces of a typical dorsal segmental artery could be identified with certainty in the sections. It seems improbable that this and other similar vessels are actually incomplete, more probable that they are complete and that in places are so small and indistinctly differentiated from the mesenchyma that the connecting portions have been overlooked. The lateral branches of the second, third, and fourth dorsal segmental arteries unite with the anterior cardinal vein; those of the remaining dorsal segmental arteries join the

posterior cardinal vein. The fifth and sixth dorsal segmental arteries are shown in plate 6, figure 2. The seventh and eighth are similar to the fifth and sixth; in addition the eighth presents a bulbous swelling which lies against the lateral wall of the medullary tube. The ninth is almost typical, but its ventral branch is either lacking or indistinguishable. The tenth to seventeenth show bulbous swellings (similar to that of the eighth) of various size extending longitudinally along the neural tube. At some places these swellings extend toward one another and probably form the longitudinal anastomoses along the neural tube, which Felix[10] has indicated are present in the Robert Meyer embryo No. 300. Evans[7] has shown that such anastomoses exist in the form of a distinct plexus in the chick embryos. In my specimen anastomoses very probably are present, for what appear to be networks of endothelial cells connecting adjacent segmental arteries are distinguishable in many places.

The eighteenth to twenty-fourth dorsal segmental arteries seem to be less well developed. They can only be followed with difficulty, owing to their smallness and to the plane in which the sections are cut. Their origins from the aorta, however, are very apparent.

*Ventral Segmental Arteries.*—The ventral segmental arteries are paired vessels, but they are found only in the lower segments of the body and their segmental arrangement is not so definite as that of the dorsal branches. According to Evans[8] there is originally a ventral artery for each segment, but those of the upper body-segments degenerate very early. The upper ones together constitute a row of vitelline arteries, which later, by fusion of the individuals of certain pairs, give rise to the unpaired median vessels of the adult.

In my specimen there are 19 to 20 pairs of ventral segmental arteries in all, including those which go into the formation of the umbilical arteries and which should undoubtedly be classified as ventral segmentals. They extend from the seventh segment to the tail. The largest ones are placed opposite the seventeenth and eighteenth somites. All of the ventral segmental arteries pass ventrally along the wall of the digestive tract. Anastomoses, such as Felix has shown, can be made out in certain places. Most of the branches above the nineteenth segment can be traced to the yolk stalk and sac, where they become larger and enter into an extensive plexus. Those below the nineteenth segment go into the formation of the umbilical arteries, as described below.

*Lateral Segmental Arteries.*—Lateral segmental arteries are found opposite the twelfth to nineteenth segments. They are small vessels, directed laterally at right angles to the longitudinal exis of the aorta. They can be traced as far out as the nephric system. In several places connections can be observed between them and the termination of the medial tributary of the posterior cardinal vein (the beginning median subcardinal veins as described by Evans). I have been unable to determine whether or not lateral segmental vessels exist below the nineteenth segment, owing to the plane of section and to their indefiniteness.

*Terminal Branches.*—The dorsal aortæ terminate in the tail of the embryo by breaking up into distinct networks of small vessels. These plexuses probably

represent the caudal arteries in their earliest stage, but as yet they can not be said to exist as definite arteries. I have referred to one of them in plate 5, figure 2, as the "arterial plexus of the tail."

*Arteriæ Umbilicales.*—The umbilical arteries are formed by the union of the lower ventral segmental arteries, including all those caudal to the twentieth segment. Apparently one or two small capillary twigs from the tail plexus also enters into its formation. Anastomoses between the individual roots of the artery are apparent, thus giving rise to a network. In plate 5, figure 2, the network is represented diagrammatically, since I found it unprofitable to attempt to plot them with any degree of accuracy. In the first part of their course, where they lie on either side of the allantoic duct, the umbilical arteries are small and indistinct. Soon, however, they rapidly enlarge and fuse together to form a large trunk within the body-stalk. Reaching the chorion, the single umbilical artery breaks up into a number of branches, which, after repeated division, terminate as capillaries in the substance of the villi. The relation of the allantoic duct to the fork formed by the fusing umbilical arteries has been described above.

## CŒLOM.

The cœlom is represented by a continuous elongated cavity which is in wide communication with the extra-embryonic cœlom. Already it can be divided into two distinct parts, the pericardial and the pleuro-peritoneal cavities. The first of these surrounds the heart, except where the heart is attached by means of its sinus venosus behind and by its truncus arteriosus above.

### PERICARDIAL CAVITY.

The pericardial cavity reaches its highest point in the region of the bulbus cordis. Its form is shown in plate 6, figures 3 and 4. It is bounded dorsally by the pharynx and septum transversum, ventrally and laterally by the thin body-wall. Its floor is formed by the septum transversum. The floor is deficient on either side dorsally where the transverse septum is not yet complete, and in the space between it and the posterior body-wall the pericardial cavity establishes its communication on either side with the pleuro-peritoneal cavity which lies below.

### PLEURO-PERITONEAL CAVITY.

The pleuro-peritoneal cavity is divisible into two portions, an upper and a lower. The upper is formed by two narrow limbs which unite below the yolk-stalk to form the single lower portion. The limbs of the upper portion join the pericardial cavity high up on its dorsal surface. At first they are directed dorsally and caudalward, but very soon bend directly caudalward. As viewed from behind, their median borders are not straight, but each presents two curves, the concavities of which are directed medially. Fitting into the spaces formed by these curves, as shown in plate 6, figure 4, is the lung diverticulum above and the hepatic diverticulum below. That portion of the digestive tube which I have considered as the gastric region lies opposite the constricted area between the two enlarged spaces.

Caudally the two limbs terminate by uniting below the yolk-stalk. Slightly above this level the pleuro-peritoneal cavity joins the exocœlomic cavity on either side and in front where the anterior body-wall is deficient; that is, following the line of reflection of the amnion (plate 6, fig. 3). That portion of the exocœlomic cavity which lies in front bridges across the space between the two above-described limbs. The bridge lies just above the yolk-stalk, while lying between it and the pericardial cavity are the amnion, the amnionic cavity, and the anterior pericardial wall.

The lower portion of the pleuro-peritoneal cavity lies in relation to the hind-gut dorsally and the posterior surface of the yolk-sac ventrally. On either side it is in wide communication with the exocœlomic cavity. As seen in plate 6, figure 3, its anterior surface is irregularly pitted, the cast of the irregular surface of the yolk-sac. Viewed from behind (plate 6, fig. 4), is a deep groove which marks the position of the hind-gut and dorsal mesentery. At the dorsal bend in the back of the embryo the cœlomic cavity continues caudally by means of two prolongations (plate 1, fig. 2, and plate 6, fig. 4). These lie laterally to the hind-gut and cloaca. They extend about as far caudally as the cloacal membrane. It is to be noted that these are not united together ventrally; consequently there exists in this portion of the embryo a ventral mesentery as well as a dorsal one.

## MESOTHELIUM.

The body-cavity is everywhere lined with a mesothelium which varies in character and thickness in different regions. As a rule, however, it may be said that the visceral layer is thicker than the parietal. This is not entirely true regarding that portion of the parietal pericardium which lies next to the fore-gut, for this is thicker, being composed of two to three layers of cubical cells. Since, however, this layer lies next to the fore-gut, it is in reality visceral, so that its being thick is not actually at variance with the general rule. The parietal pericardium elsewhere, *i. e.*, lining the body-wall and the pericardial surface of the septum transversum, is thin, being composed of a single layer of cubical cells. The visceral pericardium, the previously described epicardium, is formed by a single layer of cubical or rounded cells.

The parietal peritoneum, with the exception of that covering the peritoneal surface of the septum transversum, is thin and formed by a single layer of flattened cells. Where this becomes continuous with the visceral peritoneum (that is, on the dorsal mesentery) it gradually becomes thicker. The inferior surface of the septum transversum possesses a comparatively thick epithelium, being composed of three to four layers of cells. The visceral peritoneum is thick over the fore-gut and yolk-stalk, somewhat thinner over the hind-gut, and thinnest over the yolk-sac, where in most places it becomes lost as a distinct layer.

The lining of the extra-embryonic cavity, *i. e.*, the inner lining of the chorionic vesicle, is not in the form of a distinct cell layer, but appears to be made up of uncovered mesenchyma. Mitotic figures are found throughout all parts of the visceral and parietal epithelium, except in the epicardium.

## SEPTUM TRANSVERSUM.

The septum transversum is a thick plate of mesenchyma which is lined on either side with the mesothelium of the cœlomic cavity. It divides off the pericardial from the pleuro-peritoneal cavity, except posteriorly and laterally, where it is incomplete. It is placed across the body at the level of the venous end of the heart and liver diverticulum and is directed obliquely from in front dorsally and cephalad. As near as can be determined, it is placed opposite the fourth and fifth body-somites. With reference to Mall's[34] schema (Mall's fig. 400), it may be said that in position the transverse septum of my embryo lies dorsally at a corresponding position which he has shown for embryos of 2 and 4 mm., and is directed at an angle which would pass some place between those of the 2 and 4 mm. embryos.

Laterally and in front the transverse septum is attached to the body-wall. Postero-medially it is attached to the posterior body-wall by means of the sinus venosus and fore-gut. Postero-laterally its border is free and covered with mesothelium. On either side, between this border and the posterior body-wall, are the communications between the pericardial and pleuro-peritoneal cavities.

Within the substance of the transverse septum are found the following structures: Laterally, running along its attachments to the body-wall, are the umbilical veins. Extending from below, nearer the median plane, are the vitelline veins. The network formed at the terminal ends of these veins and the sinus venosus formed by their union all lie within the transverse septum. In addition is the relatively large hepatic diverticulum, which occupies a central position within the septum.

Structurally the transverse septum is composed of a dense syncytium of closely packed mesenchymal cells. At the time of fixation these cells were undoubtedly in a state of rapid growth, for mitotic figures are very abundant.

## EMBRYONIC MEMBRANES.

### AMNION.

The amnion forms a closed sac, in which lies the greater part of the embryo. It is composed of two layers of epithelium: an inner, directly continuous with the skin ectoderm, and an outer, directly continuous with the mesothelial lining of the cœlom. Between these layers is a small amount of mesenchyma.

#### LINE OF REFLECTION.

The amnion is reflected from the body of the embryo as follows: cephalad it gains attachment to the body-wall at the lower border of the pericardium; laterally the line of reflection crosses the yolk-stalk on either side and continues directly caudally along the edge of the body-wall to the body-stalk. Although directly continuous with it, the demarcation between the anterior body-wall and the amnion is clearly distinguishable because of the greater thickness of the former. The body-wall ends abruptly not only by a sudden diminution of its size, but also by giving origin to the villus-like projections described above (plate 1, fig. 2). Upon reaching the body-stalk the two lines of reflection of the amnion

approach each other, so that the dorsal surface of the body-stalk (dorsal with reference to the embryo) lies within the amnionic cavity. Some little distance beyond the tip of the tail of the embryo, at the distal end of the body-stalk, the two lines come together and complete the continuous line of reflection. The entire anterior half, a small portion of the caudal end, and the dorsum of intervening portions of the embryo and dorsum of the body-stalk lie within the amnionic cavity.

### HISTOLOGICAL STRUCTURE.

Structurally the inner layer of the amnion is composed of a single layer of cells. These are for the most part cubical or rounded in shape and lie close together, but in places where they are further spread apart they are greatly flattened. The flattening affects only the protoplasm of the cells, the nuclei remaining rounded. The cells of the outer layer are also rounded and contain rounded nuclei. They are so placed that their inner surfaces, *i. e.*, the surface which is directed toward the embryo, are all joined together along the mesenchyma, while the remaining portions of the cells are free from one another. The cells therefore project outward away from the amnion, each forming a rounded protuberance. In many places these cells are columnar or pear-shaped, the nuclei being distally placed. Between the two layers of epithelium is found a thin layer of mesenchymal tissue, which is exceedingly poor in cells.

In the region of the yolk-stalk the amnion is somewhat thicker along its line of reflection. It is greatly pitted by branching depressions from the exocœlomic cavity lined with the typical mesothelium, which, when cut in cross-sections, give the appearance of blood-vessels. Some of these depressions are shown in plate 7, figures 1 and 3. Similar pit-like depressions have also been found over the greater part of the amnion and all along its line of reflection, on the left side particularly, and on the body-stalk, where the amnion is reflected from it (plate 7, fig. 2).

Although the cœlomic depressions when seen in cross-section resemble blood-vessels at first glance, closer examination shows that they are easily distinguishable from them. The shapes of the cells of the endothelial tubes are more flattened than those of the mesothelial; the endothelial nuclei are elongated, while those of the mesothelium are rounded; and in the blood-vessels hemoglobin-bearing blood-cells are usually found, while in the cœlomic depressions they are always absent.

I shall here mention the recent work of Bremer[3] regarding the formation of the earliest blood-vessels in man. This author finds funnel-shaped growths of the surface mesothelium in the yolk-sac and in the body-stalk, which he believes give rise to a network of blood-vessels. That the pit-like depressions which I have just described resemble in a way the funnel-shaped ingrowths of Bremer's description is apparent. Judging from Bremer's figures, the pits in my specimen are undoubtedly larger and more numerous, but it must be noted that my embryo is somewhat older than any of those which Bremer describes. I am, however, unable to make out any evidences of blood-vessel formation taking place from these depressions. No definite connections are apparent anywhere between the depressions and the blood-vessels.

## CHORION.

### CHORIONIC MEMBRANE.

The chorion forms a large vesicle, within the cavity of which lies the embryo. It is composed of two continuous layers, an inner mesenchymal and an outer epithelial. The outer surface of the chorionic membrane is covered with chorionic villi over which the epithelium is continuous. On the side adjacent to the attachment of the body-stalk the surface of the chorion is free from villi and here both epithelium and mesenchymal layers are reduced in thickness. This side is entirely collapsed and thrown into a large fold, as shown in text-figure 1.

### CHORIONIC VILLI.

The chorionic villi are of variable size. The largest ones, which measure from 1.1 mm. to 1.3 mm. in height, belong to the chorion frondosum. At their bases they are ordinarily smaller than farther out. One of this type is shown in plate 7, figure 4. It divides dicrotomously into two large stalks. These again divide and extend out almost to the end as large, stout trunks. The terminal branches are short and form either rounded or pointed projections. Smaller villi are found in between the larger ones, but they are few in number.

### HISTOLOGICAL STRUCTURE.

Histologically the chorion and its villi are quite similar. The epithelium which covers the underlying mesenchyma is composed of two distinct layers of cells. The outer of these is made up of cuboidal cells without distinct boundaries. The protoplasm of its cells is finely granular and vacuolated and stains deeply with eosin. Its free surface is in most places covered with a prickle-process border as described by Grosser[14], giving very much the appearance of cilia. In no place, however, could definite cilia be demonstrated. The nuclei are of irregular rounded or oval contour and deeply staining. Beneath this layer is the basal layer of cells, the so-called "Langhan's layer," which is visibly separable from the outer layer. Its cells are also cuboidal, but the protoplasm in most cells is clearer, more distinctly vacuolated, and not so deeply stained. The nuclei are of rounded shape and are clearer than those of the outer layer. The basal surface of this layer rests directly upon the mesenchyma, there being no basement membrane present. It is irregular, owing to the varied shapes of the ends of the cells, which in places seem to be drawn out into processes which unite with those of the mesenchymal cells.

In connection with the syncytial layer are to be described the so-called giant cells and cell islands. The former are masses of deeply staining protoplasm containing several nuclei. They vary in size and in the number of nuclei contained. As pointed out by Frassi[11] they are merely portions of the syncytium, which in many cases are still attached to the epithelium. In my specimen such attachments are readily observable.

The cell-islands are masses of trophoblastic cells which, as stated by Grosser[13], are always attached to villi. They represent portions of the trophoblast which have failed to become spread out over the villi. They are made up of large decidual-like cells of polygonal shape and distinct boundaries. Their nuclei are usually shriveled and their protoplasm is clear and vacuolated. In the centers of these cell-islands are evidences of degeneration. The cells are much broken up, nuclei are very

dark and irregular, and considerable fibrin is present. The so-called "cell columns" (Grosser) are similar masses of cells, by means of which the villi are attached to the decidua. They are numerous at the ends of the villi. The intervillous spaces are remarkably free from maternal blood-corpuscles, only a few being found.

The stroma of the chorion and its villi is made up of a network of loose mesenchyma. Its cells are stellate in shape and finely granular; the nuclei are oval, rich in chromatin, and contain nucleoli. In places are to be found greatly elongated nuclei, much resembling those of smooth muscle-fibers. These are found just beneath the Langhan's layer.

Throughout the interspaces of the mesenchyma are to be found blood-vessels of various sizes, filled in many instances with blood elements; also the so-called "Hofbauer" cells (Grosser[13]) with highly vacuolated protoplasm and large nuclei; these appear in the body-stalk as well and occasionally in blood-vessels.

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## EXPLANATION OF PLATES.

### Plate 1.

1. Model of whole embryo viewed from left side. The body-wall in the region of the heart and septum transversum has been dissected away. At X, pericardo-pleural passage. ×80 diameters.
2. Same, viewed from in front. ×80 diameters.

### Plate 2.

1. Brain and portion of neural tube. In light numbers are indicated brain and spinal-cord neuromeres, numbered from the mesencephalon backward. The black lines and numbers indicate the position of the body-segments. It is to be noted that the neuromeres are intersegmental in position. ×80 diameters.
2. Pharynx, cut longitudinally and left half viewed from medial surface. ×133 diameters. From a model by Mr. L. H. Rutledge.
3. Same, viewed from right side with integument removed. ×133 diameters.
4. Ventral surface of same. ×133 diameters.

### Plate 3.

1. Heart as seen from dorsal surface. From a model by Mr. Florien Vaughn. ×166 diameters.
2. Cavity of the heart as seen from dorsal surface. From a model by Mr. Florien Vaughn. ×166 diameters.
3. Model of fore-gut viewed from left side. ×266 diameters.
4. Ventral surface of same. ×266 diameters.

### Plate 4.

1. Portion of section 94 showing beginning of heart sinusoid in ventricular portion of heart. ×600 diameters
2. Portion of section 165 passing through twelfth somite. ×325 diameters.
3. Portion of section 116. ×600 diameters.
4. Nephric system of left side viewed from medial surface. From wax reconstruction. ×125 diameters.

### Plate 5.

1. Graphic reconstruction of venous system. ×80 diameters. At X, junction of large veins with sinus venosus.
2. Graphic reconstruction of arterial system. ×80 diameters. Between X and X the dorsal aortae have fused, forming a single vessel.

### Plate 6.

1. The sinus venosus and entering veins. From a wax reconstruction by Mr. T. F. Wheeldon. ×120 diameters.
2. Wax reconstruction showing relations of the dorsal segmental arteries. Viewed from behind forward. ×120 diameters.
3. Wax reconstruction of the cœlomic cavity viewed from in front. ×63 diameters.
4. Same, viewed from behind. ×63 diameters.

### Plate 7.

1. Portion of section 127, showing cœlomic depressions in region of septum transversum and amnion. ×400 diameters.
2. Portion of section 269, showing cœlomic depressions in region of body-stalk. ×275 diameters.
3. Cast of cœlomic depressions in region of transverse septum. The irregular depressions are in places branched, as shown on the left side of figure. ×215 diameters.
4. Villus from chorion frondosum. From a wax reconstruction by Mr. H. L. Houchins. ×60 diameters.
5. Graphic reconstruction of left half of embryo, viewed from medial side. Numerous models have been used in making the drawing. ×60 diameters.

### Plate 8.

1–12. Cross sections of embryo. ×40 diameters.

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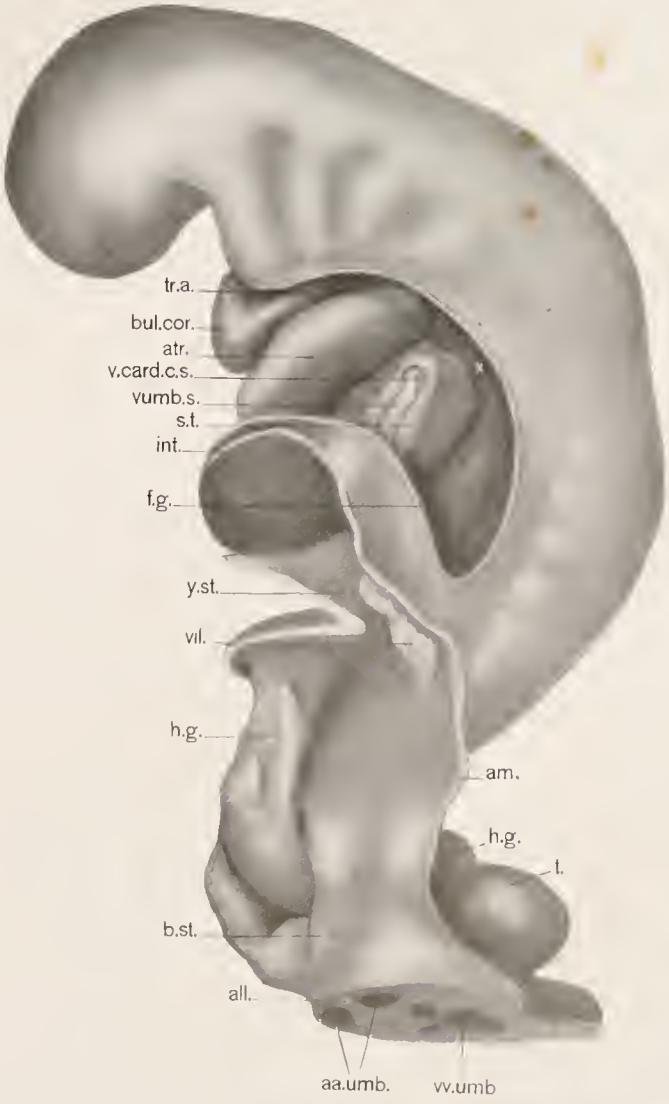
| 3. Section 63. | 6. Section 123. | 9. Section 180. | 12. Section 254. |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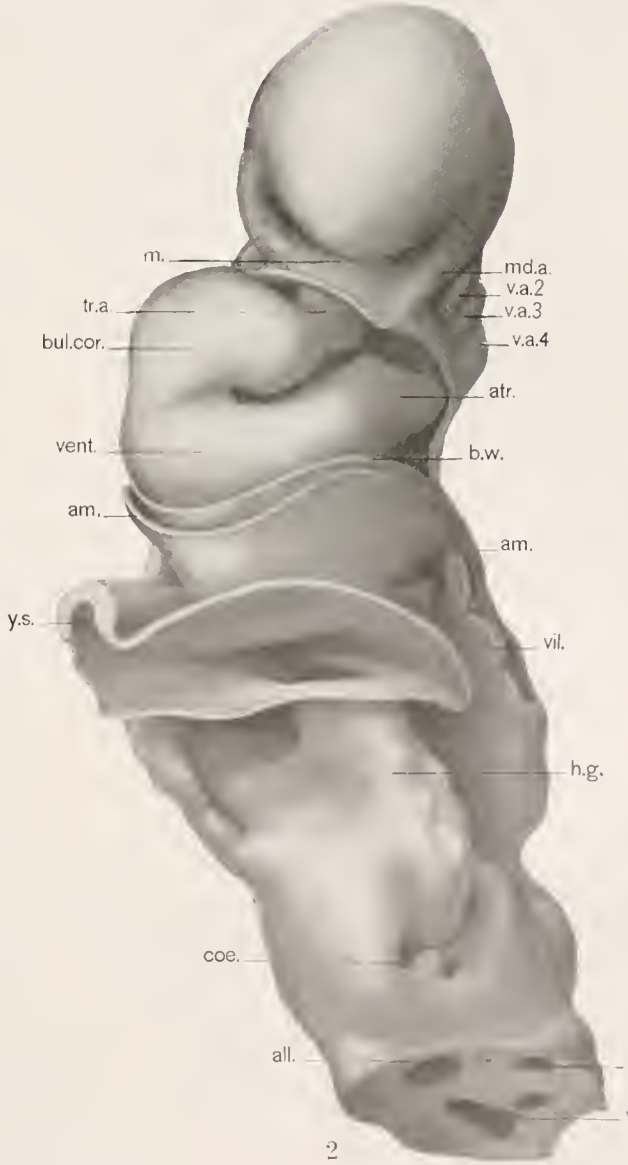
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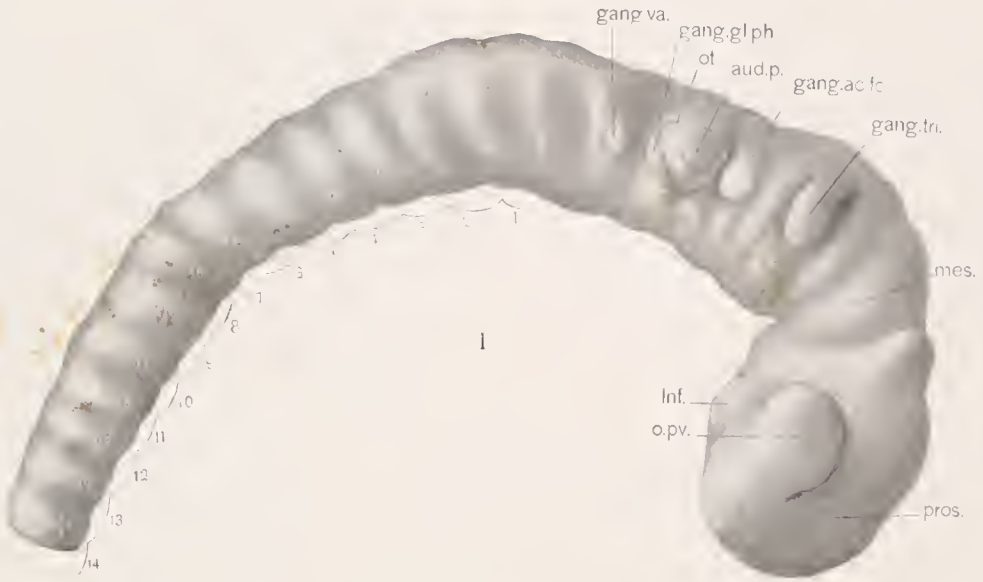
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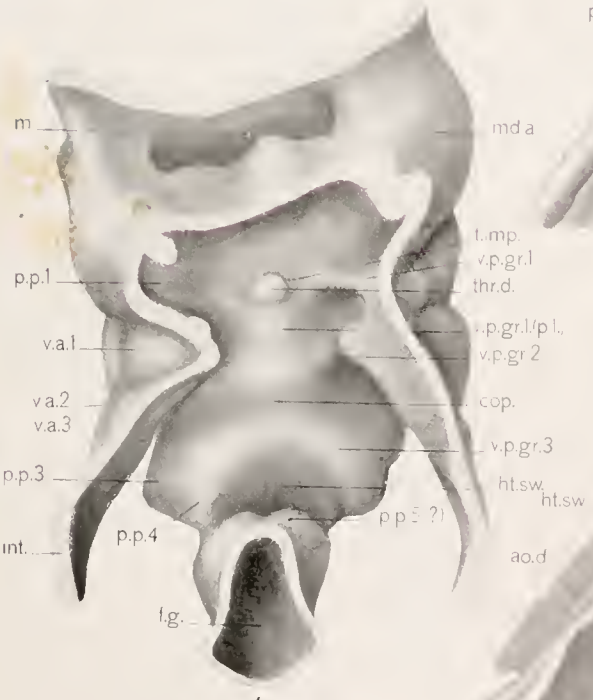
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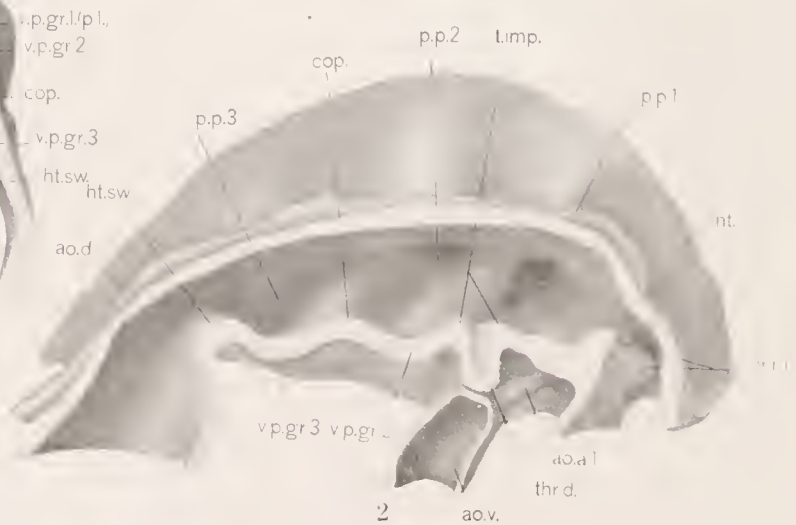
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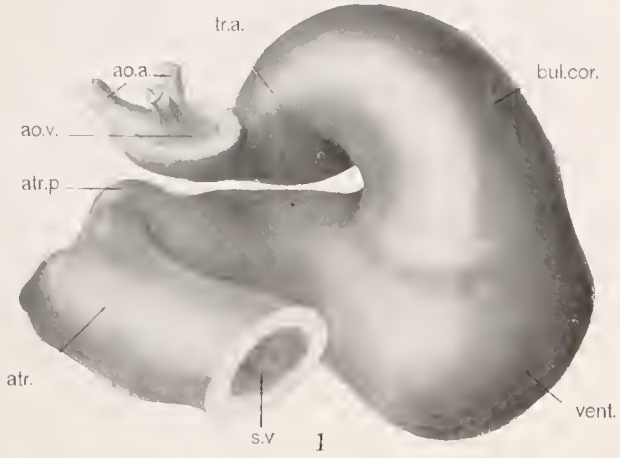
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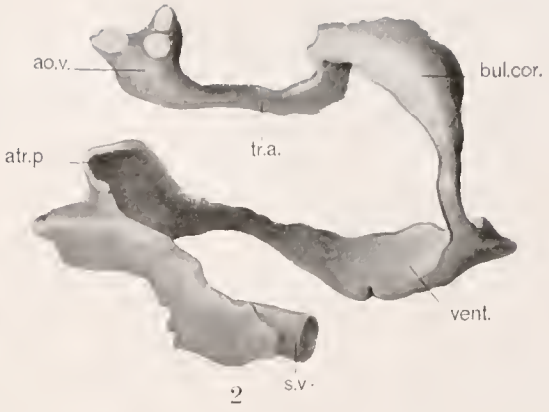
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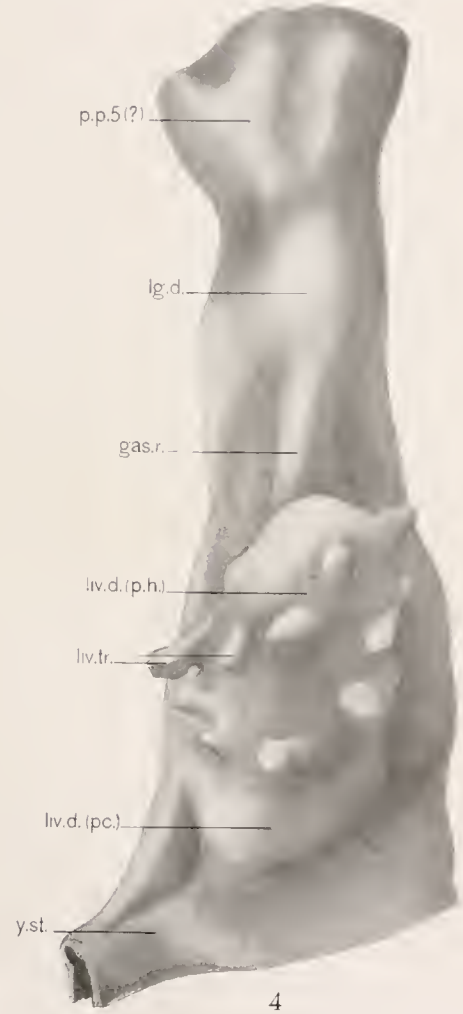
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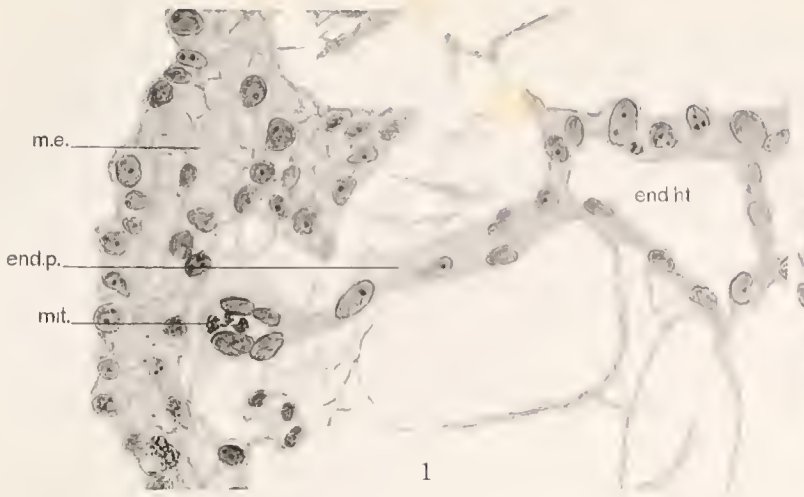


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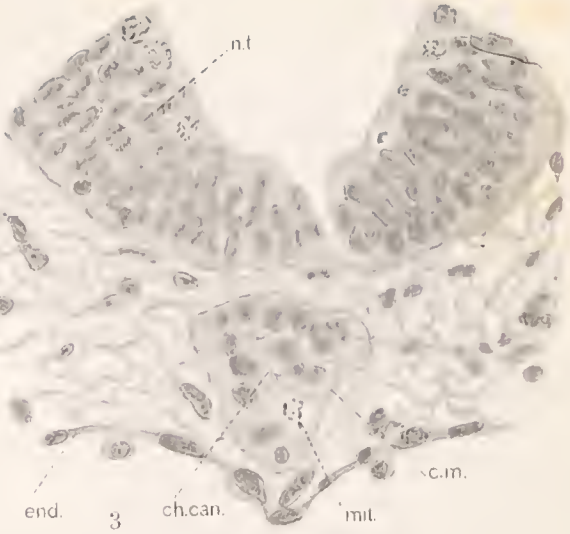


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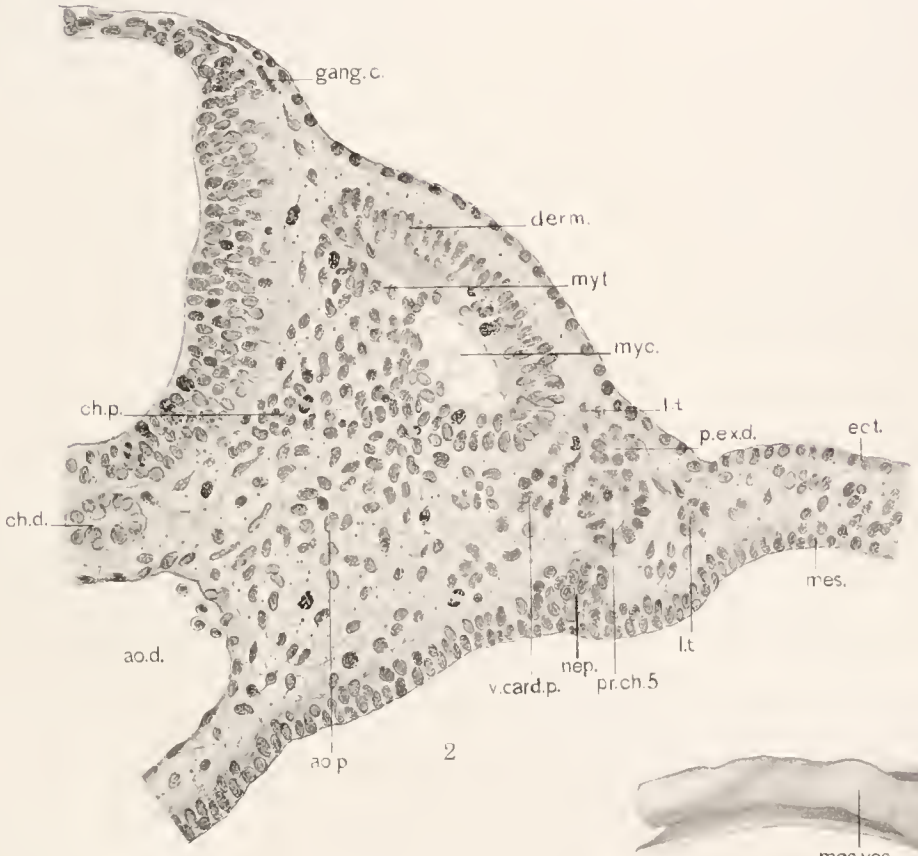


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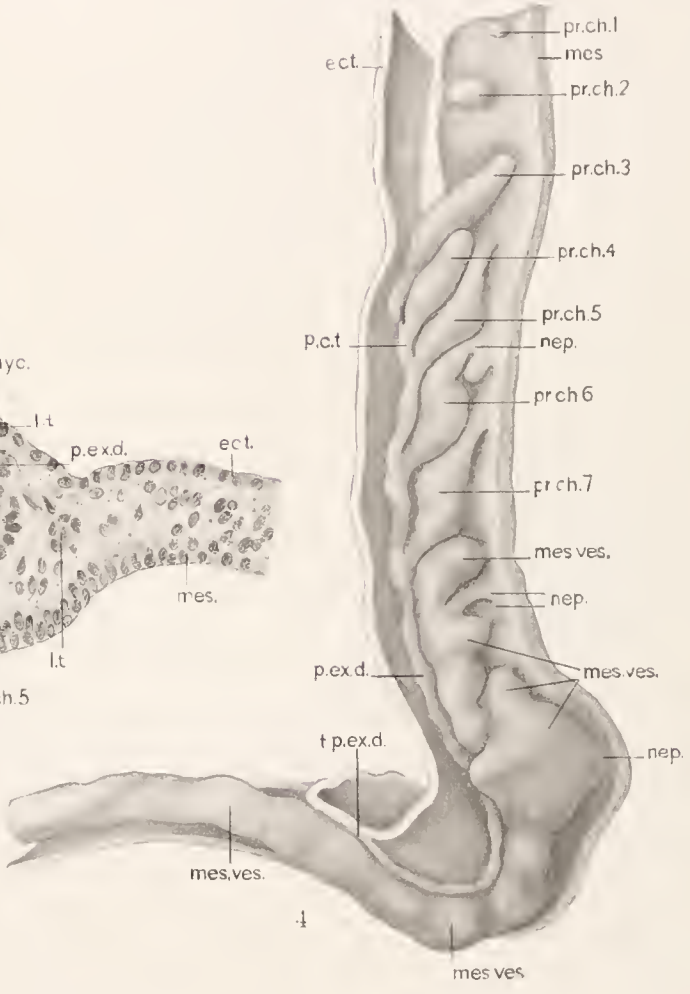


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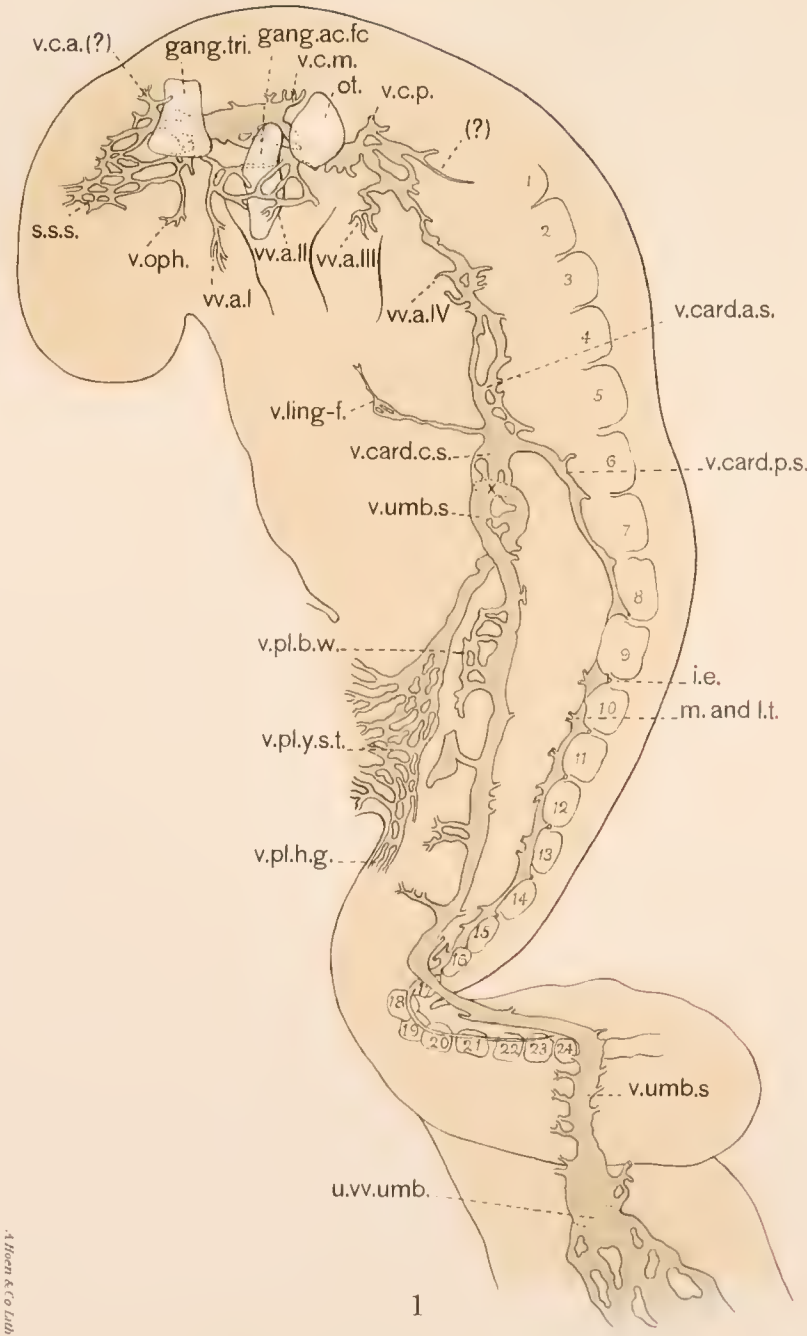


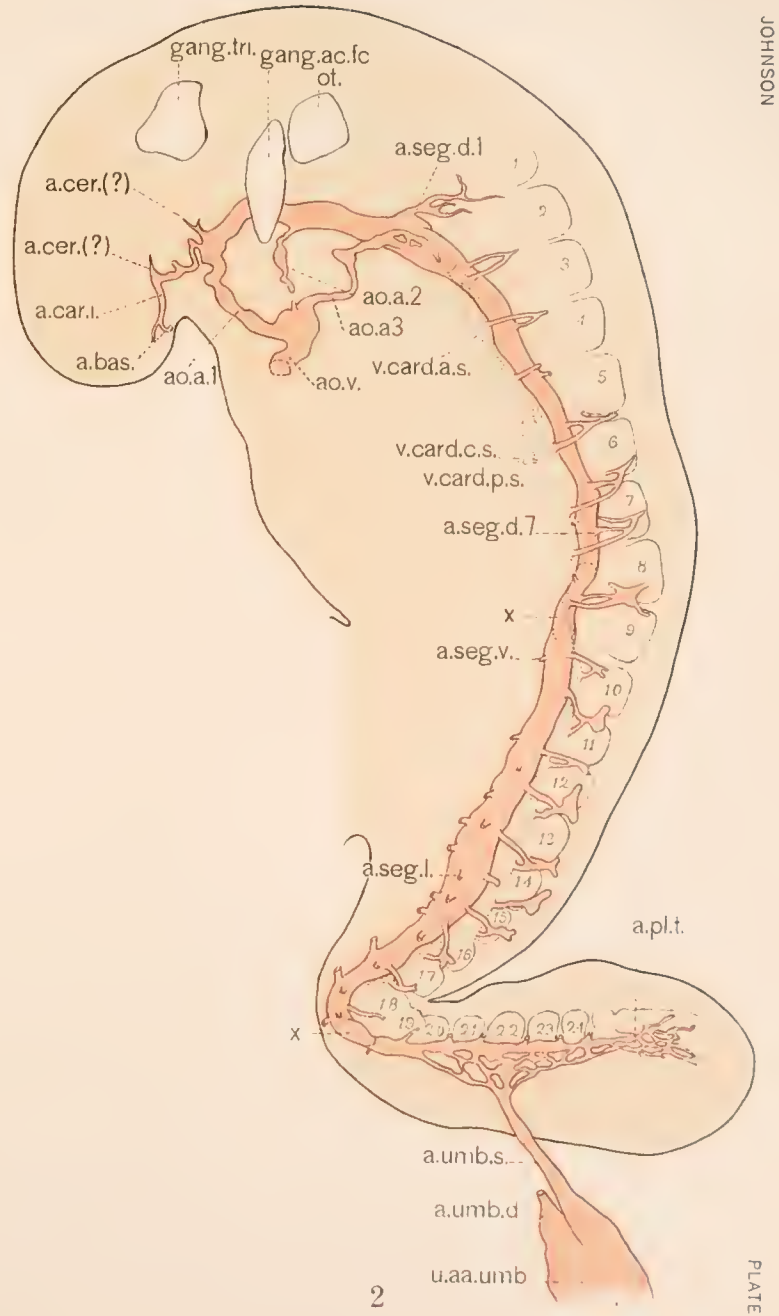




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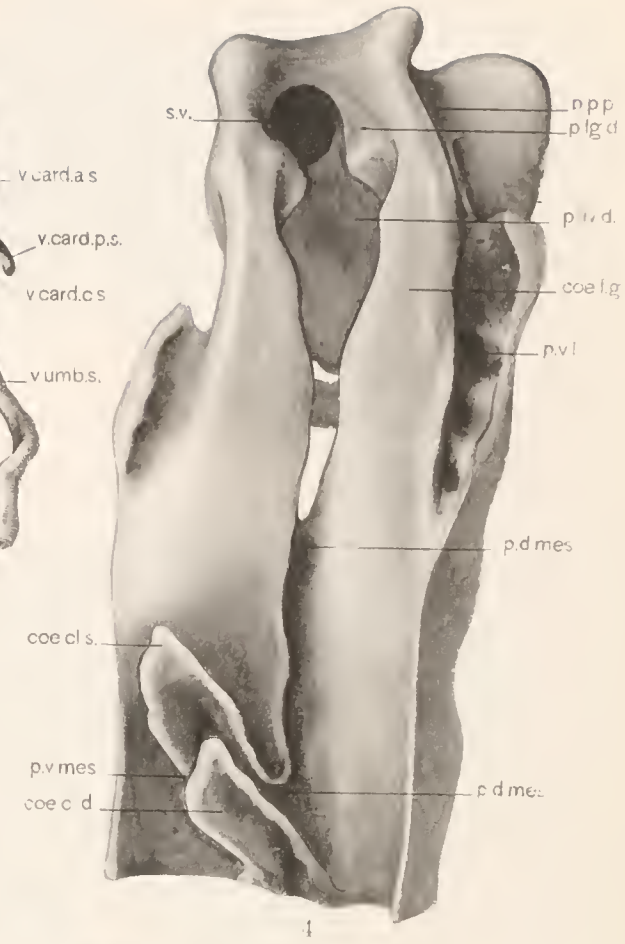
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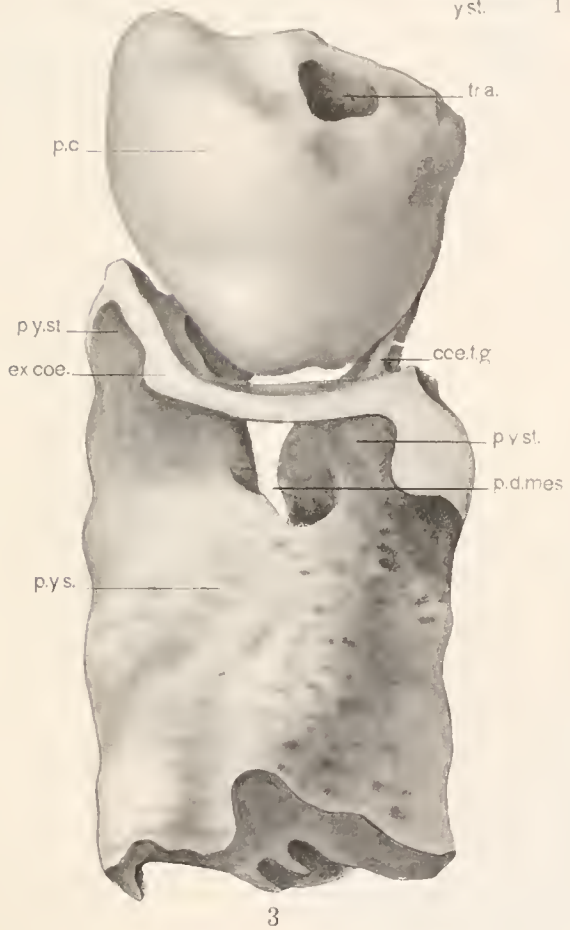
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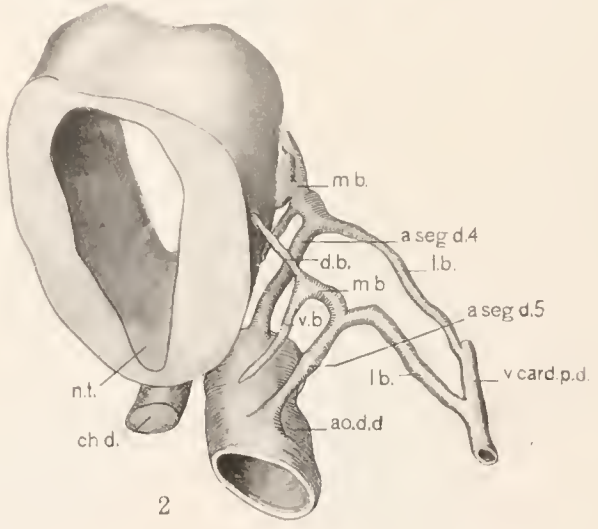
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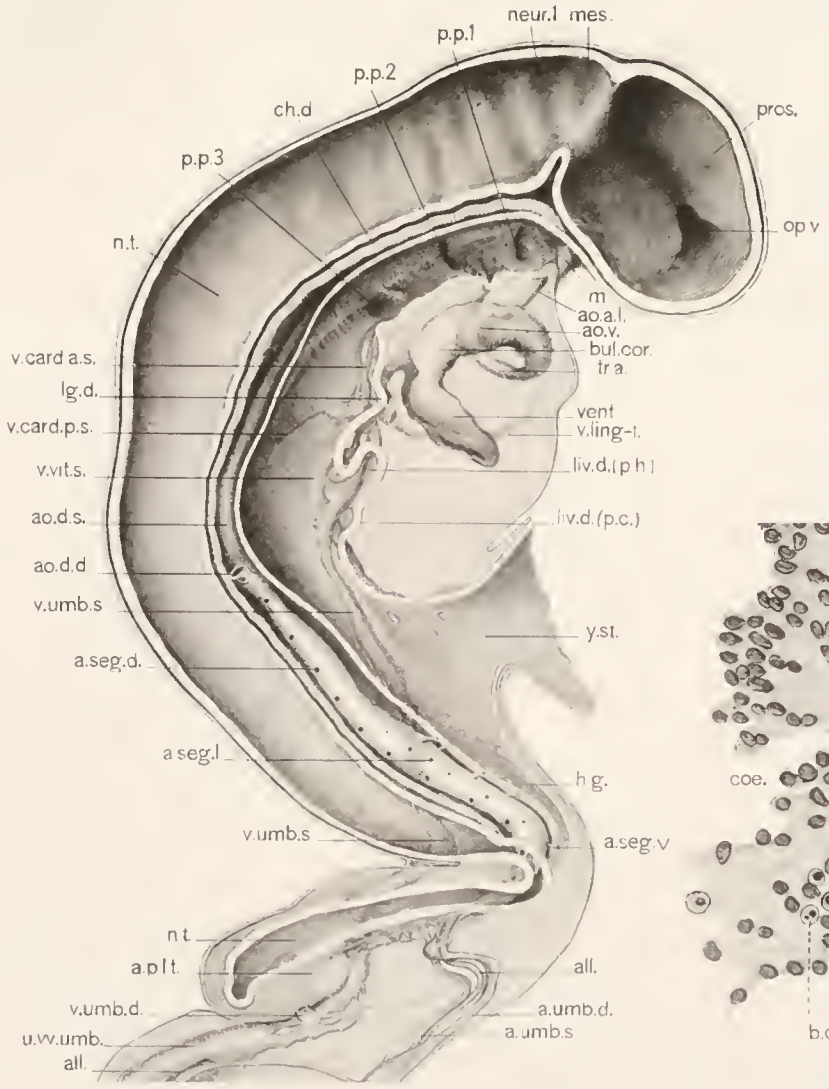


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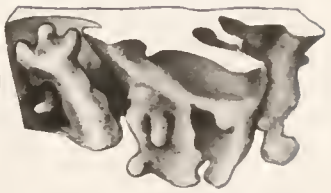

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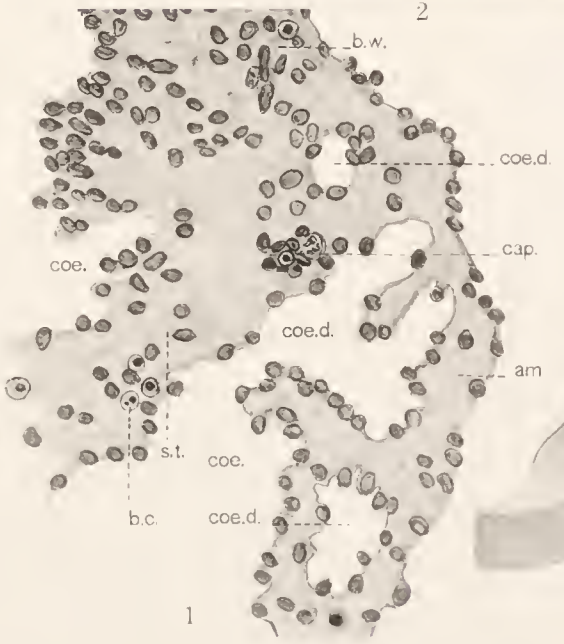


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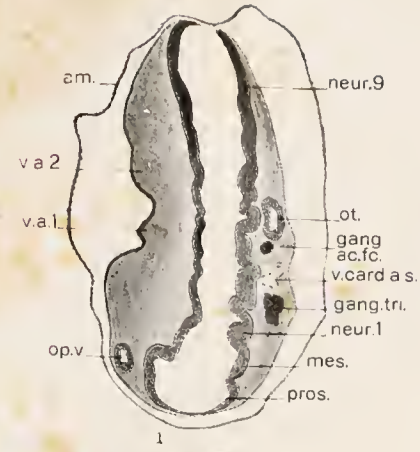

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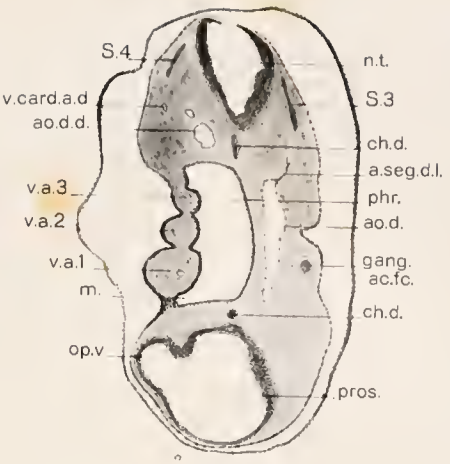

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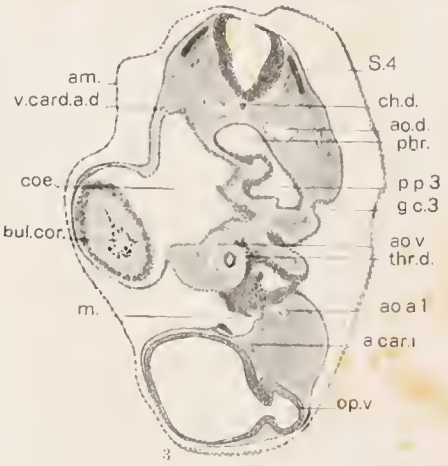

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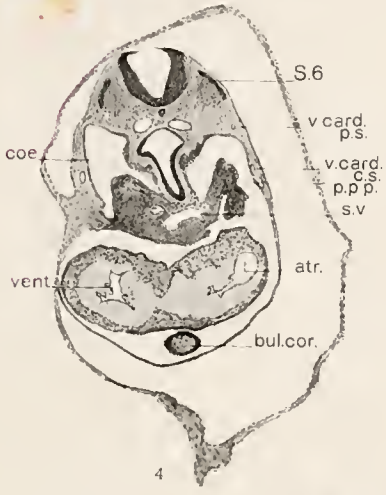

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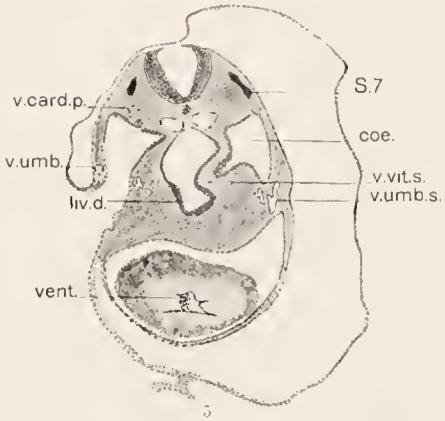

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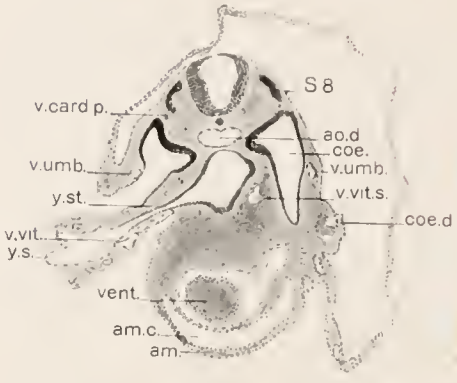

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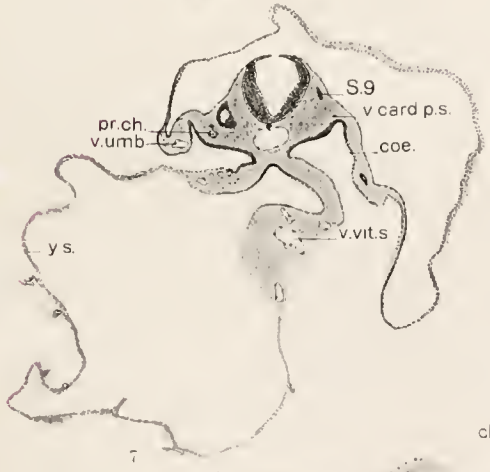

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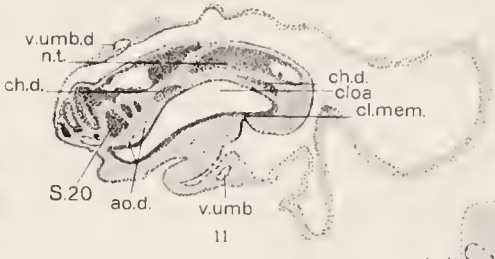

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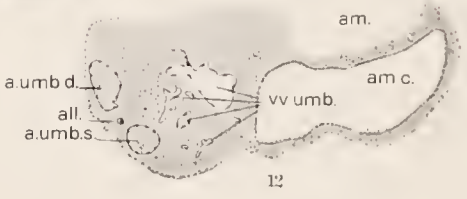

12

# ABBREVIATIONS.

| | |
|---|---|
| a. bas., | a. basilaris. |
| a. cer., (?) | a. cerebri (?). |
| a. car. i., | a. carotis interna. |
| a. seg. d., | a. segmentalis dorsalis. |
| a. seg. l., | a. segmentalis lateralis. |
| a. seg. v., | a. segmentalis ventralis. |
| a. umb., | a. umbilicalis. |
| a. umb. d., | a. umbilicalis dextra. |
| a. umb. s., | a. umbilicalis sinistra. |
| aa. umb., | aa. umbilicales. |
| all., | allantois. |
| am., | amnion. |
| am. c., | amnionic cavity. |
| ao. d., | aorta dorsalis. |
| ao. d. d., | aorta dorsalis dextra. |
| ao. d. s., | aorta dorsalis sinistra. |
| ao. v., | aorta ventralis. |
| ao. a., | aortic arch. |
| ao. p., | aortic process of sclerotome. |
| a. pl., t., | arterial plexus of tail. |
| atr. p., | atrial projection. |
| atr., | atrium. |
| aud. p., | auditory pit. |
| b. c., | blood-corpuscles. |
| b. v., | blood-vessels. |
| b. st., | body-stalk. |
| b. w., | body-wall. |
| bul. cor., | bulbus cordis. |
| cap., | capillary. |
| cap. pl., | capillary plexus. |
| c. int., | caudal intestine. |
| ch. d., | chorda dorsalis. |
| ch. can., | chordal canal. |
| ch. p., | chordal process of sclerotome. |
| cloa., | cloaca. |
| cl. men., | cloacal membrane. |
| c. m., | cuticular membrane. |
| coe., | cœlom. |
| coe. cloa. d., | cœlom on right side of cloaca and caudal intestine. |
| coe. cloa. s., | cœlom on left side of cloaca and caudal intestine. |
| coe. d., | cœlomic depressions. |
| coe. f. g., | cœlom surrounding fore-gut. |
| cop., | copula. |
| d. b., | dorsal branch of medial limb of a. seg. d. |
| ect., | ectoderm. |
| end. ht., | endothelial heart. |
| end. p., | endothelial process. |
| end., | endothelium. |
| ex. coe., | exocœlomic cavity. |
| f. g., | fore-gut. |
| gang. ac. fc., | ganglion acustico-facialis. |
| gang. c., | ganglion crest. |
| gang. gl. ph., | ganglion n. glossopharyngei. |
| gang. tri., | ganglion n. trigemini. |
| gang. va., | ganglion n. vagi. |
| gas. r., | gastric region. |
| g. c., | gill-cleft. |
| ht. sw., | heart swelling (Grosser). |
| h. g., | hind-gut. |
| inf., | infundibulum. |
| int., | integument. |
| i. e., | intersegmental enlargement of v. card. p. |
| l. b., | lateral branch of a. seg. d. |
| l. t., | lateral tributary of v. card. p. |
| liv. d. (p. c.), | liver diverticulum (pars cystica). |
| liv. d. (p. h.), | liver diverticulum (pars hepatica). |
| liv. tr., | liver trabeculae. |
| lg. d., | lung diverticulum. |
| md. a., | mandibular arch. |
| m. b., | medial branch of a. seg. d. |
| m. and l. t., | medial and lateral tributaries of v. card. p. |
| m. c., | mesocardium. |
| mes., | mesencephalon. |
| mes. ves., | mesonephric vesicles. |
| meso., | mesothelium. |
| mit., | mitotic figure. |
| m., | mouth. |
| m. c., | myocpicardium. |
| myc., | myocoele. |
| myt., | myotome. |
| nep., | nephrostome. |
| n. t., | medullary tube. |
| neur., | neuromere (1–21). |
| op. v., | optic vesicle. |
| ot., | otocyst. |
| or. m., | oral membrane remnants. |
| p. c., | pericardial cavity. |
| p. d. mes., | position of dorsal mesentery. |
| p. p. p., | pleuro-pericardial passage. |
| p. p., | pharyngeal pouch. |
| phr., | pharynx. |
| p. ex. d., | primary excretory duct. |
| p. c. t., | principal collecting tubule. |
| p. lg. d., | position of lung diverticulum. |
| pr. ch., | pronephric chamber. |
| proc. inv., | proctodeal invagination. |
| pros., | prosencephalon. |
| p. v. mes., | position of ventral mesentery. |
| p. y. s., | position of yolk-sac. |
| p. y. st., | position of yolk-stalk. |
| selt., | sclerotome. |
| s. t., | septum transversum. |
| s. s. s., | sinus sagittalis superior. |
| s. v., | sinus venosus. |
| s., | somite (1–22). |
| t., | tail. |
| tear, | tear in tissue. |
| thr. d., | thyroid diverticulum. |
| tr. a., | truncus arteriosus. |
| t. imp., | tuberculum impar. |
| t. p. ex. d., | termination of primary excretory duct. |
| u. aa. umb., | union of aa. umbilicales. |
| u. vv. umb., | union of vv. umbilicales. |
| v. card. a. d., | v. cardinalis anterior dextra. |
| v. card. a. s., | v. cardinalis anterior sinistra. |
| v. card. c. d., | v. cardinalis communis dextra. |
| v. card. c. s., | v. cardinalis communis sinistra. |
| v. card. p. d., | v. cardinalis posterior dextra. |
| v. card. p. s., | v. cardinalis posterior sinistra. |
| v. c. a., (?) | v. cerebri anterior (?). |
| v. c. m., | v. cerebri media. |
| v. c. p., | v. cerebri posterior. |
| v. ling-f., | v. linguo-facialis. |
| v. oph., | v. ophthalamicus. |
| v. umb. d., | v. umbilicalis dextra. |
| v. umb. s., | v. umbilicalis sinistra. |
| v. vit. d., | v. vitellina dextra. |
| v. vit. s., | v. vitellina sinistra. |
| vv. a., | veins of visceral arches (I–IV). |
| vv. umb., | venæ umbilicales. |
| v. pl. b. w., | venous plexus of body-wall. |
| v. pl. h. g., | venous plexus of hind-gut. |
| v. pl. y. st., | venous plexus of yolk-stalk. |
| v. b., | ventral branch of medial limb of a. seg. d. |
| v. p. gr., | ventral pharyngeal groove (1–3). |
| v. p. gr. 1 (p. l.), | ventral pharyngeal groove 1-posterior limb. |
| vent., | ventricular portion of heart. |
| vil., | villus-like projections of body-wall. |
| v. a., | visceral arch (1–3). |
| y. s., | yolk-sac. |
| y. st., | yolk-stalk. |

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